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L12 ANSWER 2 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 138:384046 CA
TI A double staining flow cytometric assay for the detection of steroid induced apoptotic leukocytes in common carp (Cyprinus carpio)
AU Saha, Nil Ratan; Usami, Takeshi; Suzuki, Yuzuru
CS Graduate School of Agricultural and Life Sciences, Fisheries Laboratory, The University of Tokyo, Hamana-gun, Shizuoka, 431-0211, Japan
SO Developmental & Comparative Immunology (2003), 27(5), 351-363
CODEN: DCIMDQ; ISSN: 0145-305X
PB Elsevier Science B.V.
DT Journal
LA English
AB Steroid hormones play an important role in the regulation of the immune system through different ways. In this in vitro study, the effects of steroid hormones on the **apoptosis** of leukocytes were evaluated to understand the involvement of this process in the immunocompetence of common carp. Prior to the investigation, a double staining flow cytometric assay using fluorescein diacetate (FDA), which reacts with esterases of viable cells, and propidium iodide (PI), an acid dye that binds with nuclear DNA, was established. FDA and PI neg. cells were regarded as apoptotic. The FDA-PI technique is comparable to the Annexin V-PI technique and can be used in the quantification of the **apoptosis** of fish leukocytes accurately. The results suggest that the disappearance of esterases and externalization of **phosphatidylserine** (PS) may be common to many apoptotic pathways. Cells collected from peripheral blood, spleen, head kidney, and thymus were cultured for 16 h either in the absence or presence of steroid hormones, i.e. cortisol (F), testosterone, 11-ketotestosterone, and estradiol-17.beta., and analyzed by flow cytometry followed by the FDA-PI method. Results showed that F induced **apoptosis** in leukocytes from blood and other lymphoid organs suggesting the role of F as an immune regulator. The participation of sex steroids to the immunocompetence of carp was not found, since they did not induce **apoptosis** of leukocytes in any organ.
RE.CNT 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 7 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 138:217707 CA
TI Annexin V-CLIO: a nanoparticle for detecting **apoptosis** by MRI
AU Schellenberger, Eyk A.; Bogdanov, Alexei, Jr.; Hogemann, Dagmar; Tait, Jonathan; Weissleder, Ralph; Josephson, Lee
CS Massachusetts General Hospital, Charlestown, MA, 02129, USA
SO Molecular Imaging (2002), 1(2), 102-107
CODEN: MIOMBP; ISSN: 1535-3508
PB MIT Press
DT Journal
LA English
AB Annexin V, which recognizes the **phosphatidylserine** of apoptotic cells, was conjugated to crosslinked iron oxide (CLIO) nanoparticles, a functionalized superparamagnetic prepn. developed for target-specific magnetic resonance imaging (MRI). The resulting nanoparticle had an av. of 2.7 annexin V proteins linked per CLIO nanoparticle through disulfide bonds. Using camptothecin to induce **apoptosis**, a mixt. of Jurkat T cells (69% healthy and 31% apoptotic) was incubated with annexin V-CLIO and was applied to magnetic columns. The result was an almost complete removal of the apoptotic cells (>99%). In a phantom MRI expt., untreated control cells (12% apoptotic cells, 88% healthy cells) and camptothecin-treated cells (65% apoptotic cells, 35% healthy cells) were incubated with either annexin V-CLIO (1.0, 0.5, and 0.1 .mu.g Fe/mL) or

with unlabeled CLIO. A significant signal decrease of camptothecin-treated cells relative to untreated cells was obsd. even at the lowest concn. tested. Unmodified CLIO failed to cause a significant signal change of apoptotic cells. Hence, annexin V-CLIO allowed the identification of cell suspensions contg. apoptotic cells by MRI even at very low concns. of magnetic substrate. Conjugation of annexin V to CLIO affords a strategy for the development of a MRI imaging probe for detecting **apoptosis**.

RE.CNT 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 8 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 138:216671 CA
TI Induction of **apoptosis** in mammalian cells by cadmium and zinc
AU Waetjen, Wim; Haase, Hajo; Biagioli, Marta; Beyersmann, Detmar
CS Department of Biology and Chemistry, University of Bremen, Bremen, Germany
SO Environmental Health Perspectives Supplements (2002), 110(5), 865-867
CODEN: EHPSEO; ISSN: 1078-0475
PB National Institute of Environmental Health Sciences
DT Journal
LA English
AB In various mammalian cells, two group IIb metals, cadmium and zinc, induce several morphol. and biochem. effects that are salient features of programmed cell death. In C6 rat glioma cells, cadmium caused externalization of **phosphatidylserine**, breakdown of the mitochondrial membrane potential, activation of caspase-9, internucleosomal DNA fragmentation, chromatin condensation, and nuclear fragmentation. In NIH3T3 routine fibroblasts, cadmium-induced **apoptosis** was inhibited by overexpression of the antiapoptotic protein Bcl-2. Cadmium-induced DNA fragmentation in C6 cells was independent of inhibition of protein kinase A (PKA), protein kinase C (PKC), mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase, Ca-calmodulin-dependent protein kinase, and protein kinase G. Zinc at moderate concns. (10-50 .mu.M) protected against programmed cell death induced by cadmium, whereas deprivation of zinc by the membrane-permeable chelator N,N,N',N'-tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN) caused cell death with features characteristic of **apoptosis**. On the other hand, at elevated extracellular levels (150-200 .mu.M), zinc alone caused programmed cell death in C6 cells. Zinc-induced **apoptosis** was independent of inhibition of PKA, PKC, guanylate cyclase and MAPK, but it was suppressed in the presence of 100 .mu.M lanthanum chloride.

RE.CNT 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 10 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 137:244196 CA
TI The dynamic process of **apoptosis** analyzed by flow cytometry using annexin-V/propidium iodide and a modified in situ end labeling technique
AU Span, L. F. R.; Pennings, A. H. M.; Vierwinden, G.; Boezeman, J. B. M.; Raymakers, R. A. P.; de Witte, T.
CS Department of Hematology, University Medical Center Nijmegen, Nijmegen, 6500 HB, Neth.
SO Cytometry (2002), 47(1), 24-31
CODEN: CYTODQ; ISSN: 0196-4763
PB Wiley-Liss, Inc.
DT Journal
LA English
AB To study the apoptotic process in time, we used the following flow cytometric (FCM) techniques: **phosphatidylserine** (PS) translocation by Annexin-V (AnV), DNA fragmentation by in situ end labeling (ISEL), and propidium iodide (PI) staining. Because PS translocation is assumed to be an early feature of programmed cell death

(PCD), we questioned if AnV positivity implies inevitable cell death. **Apoptosis** was induced in Jurkat cells by .gamma.-irradn., incubation with camptothecin (CPT), or cytosine .beta.-D-arabinofuranoside (Ara-C). At different time intervals, PCD was quantified by AnV/PI and ISEL. To analyze the influence of cell handling procedures on PCD, we applied these three FCM techniques on CD34 + bone marrow (BM) stem cells after selection and after a freeze-thaw procedure. Various AnV/PI - CD34 + fractions were cultured in a single-cell single-well (SCSW) assay. Jurkat cells under three different detrimental conditions showed essentially the same pattern of **apoptosis** in time. Initially developed AnV + /PI- cells subsequently (within 1 h) showed ISEL positivity, after which they turned into AnV + /PI+ cells with even higher levels of ISEL positivity (80-90%). Eventually, they lost some of their PI and ISEL positivity and formed the AnV + /PI+ fraction. Cell handling of CD34 + cells caused high and variable AnV + /PI- fractions (overall range 23-62%). Within total AnV + and AnV + /PI- populations, only a minority of CD34 + cells showed ISEL positivity (range 4-8% and 0.8-6%, resp.). Different fractions of AnV + /PI- CD34 + cells did have clonogenic capacity. PCD of cell suspensions in vitro can be followed accurately in time by these three FCM techniques. PS translocation is followed rapidly (within 1 h) by oligo-nucleosomal DNA fragmentation, after which cell (and nuclear) membrane leakage occurs. Detection of PS asymmetry by AnV-fluorescein isothiocyanate (FITC) is not always assocd. with (inevitable) **apoptosis**, as can be concluded from the proliferative capacity of AnV + /PI- CD34 + cells in the SCSW assay.

RE.CNT 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 11 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 137:228786 CA
TI Annexin V for flow cytometric detection of **phosphatidylserine** expression on lymphoma cells undergoing **apoptosis**
AU Peng, Liming; Jiang, Hong; Chris, Bradley
CS Department of Laboratory Medicine, The First Affiliated Hospital, WCUMS, Chengdu, 610041, Peop. Rep. China
SO Huaxi Yike Daxue Xuebao (2001), 32(4), 602-604, 620
CODEN: HYDXET; ISSN: 0257-7712
PB Huaxi Yike Daxue
DT Journal
LA Chinese
AB The quant. method for analyzing apoptotic and secondary necrotic cells under **apoptosis** conditions was presented. The cells of Burkitt lymphoma cell line Raji were incubated with 1.0M dexamethasone (DEX) for 2, 4, and 8 h, then stained with Annexin V-FITC (fluorescein isothiocyanate conjugated) which was used to detect the exposure of **phosphatidylserine** (PS) on the out membrane resulting from a loss of phospholipid asymmetry in the early stage of **apoptosis**, and also stained with propidium iodide which allows the anal. of secondary necrotic cells related with cell membrane and DNA damage, then apoptotic cells was quantified by flow cytometry (FMC). Furthermore, Annexin+/PI- and Annexin+/PI+ cells were sorted by fluorescence-activated cell sorter (FACS), and identified by electron microscopy (EM) and DNA gel electrophoresis. The results revealed that the percentage of apoptotic cells was increased and correlated well with incubation time ($r = 0.97$). The sensitivity of this method was shown by its detection limit 0.02%; the method was reproducible, and coeff. variance was 4.2%. The Annexin+/PI- and Annexin+/PI+ cells were identified as apoptotic and necrotic cells under EM, and DNA extd. from the Annexin+/PI- cells was characterized by "ladder pattern". Annexin V assay for analyzing apoptotic cells was specific, sensitive, accurate, reproducible, and quant. for **apoptosis** research.

L12 ANSWER 13 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 136:382452 CA

TI Measurement of DNA damage associated with **apoptosis** by laser scanning cytometry
AU Bacso, Zsolt; Eliason, James F.
CS Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, USA
SO Cytometry (2001), 45(3), 180-186
CODEN: CYTODQ; ISSN: 0196-4763
PB Wiley-Liss, Inc.
DT Journal
LA English
AB **Phosphatidylserine** (PS) binding by annexin V (AV) is an early membrane marker of **apoptosis**. Using laser scanning cytometry (LSC) and the comet assay, we showed that the DNA of AV+ cells is so highly fragmented that it cannot be quantified by the comet assay (Bacso et al.: Cancer Res 60:4623-8, 2000). The "halo" assay was used instead of the comet assay to quantify DNA damage assocd. with **apoptosis**. The LSC was used to measure both AV fluorescence and DNA damage on the same Jurkat cells following treatment with anti-Fas. The data from both sets of measurements were merged, allowing direct correlation of membrane and nuclear markers of cell death. AV+ cells had significant DNA damage detd. by the ratio between nuclear DNA and peripheral (migrated) DNA. Cells in the early and late stages of **apoptosis** could be discriminated on the basis of DNA content. In addn., it was possible to distinguish between apoptotic and necrotic cells in the AV+ propidium iodide-pos. population based on DNA content and DNA damage. The addn. of specific inhibitors for caspases-8, 9, and 3 blocked both PS externalization and DNA fragmentation, indicating these events are downstream from caspase activation. This technique allows accurate distinction between apoptotic and necrotic cells and cytometric grading of **apoptosis**.
RE.CNT 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 17 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 136:116928 CA
TI CD4 mAb induced **apoptosis** of peripheral T cells: multiparameter subpopulation analysis by flow cytometry using Attractors
AU Fishman-Lobell, J.; Tsui, P.; Reddy, M.; DiPrinzio, R.; Eichman, C.; Sweet, R. W.; Truneh, A.
CS Department of Oncology Research, SmithKline Beecham Pharmaceuticals, King of Prussia, PA, 19406, USA
SO Journal of Immunological Methods (2001), 257(1-2), 71-82
CODEN: JIMMBG; ISSN: 0022-1759
PB Elsevier Science B.V.
DT Journal
LA English
AB Studies describing the induction of **apoptosis** for CD4 mAbs do not delineate between epitope-dependent and Fc-driven epitope crosslinking induced cell death. Keliximab and clenoliximab are two CD4 mAbs that differ only in their heavy chain isotypes, being an IgG1 and a modified IgG4, resp. These antibodies suppress CD4 T cell responses in vitro and in vivo and have been in human clin. trials for the treatment of RA and asthma. Here the authors compared the apoptotic activity of these mAbs to differentiate between the contributions of epitope-dependent vs. Fc-driven epitope crosslinking induced cell death in vitro as a link to differential CD4 cell depletion in vivo. The authors developed a simple flow cytometry procedure that measures **apoptosis** within intact and compromised subpopulations of PBMCs within a few hours of culture. Attractors software was used to quantitate the percentage of apoptotic CD4 T cells, which generate reactive oxygen species (ROS), express external **phosphatidylserine** (PS) and cleaved fluorescein diacetate (FDA), within the intact and compromised lymphocyte populations. Treatment of freshly isolated PBMCs with keliximab resulted in the appearance of characteristic apoptotic condensed CD4 T cells that contained reactive

oxygen species, were annexin V pos. and had intact esterase activity. **Apoptosis** was evident within 3 h and continued throughout the 72-h culture period. In contrast, clenoliximab alone did not induce **apoptosis**. The use of multiparameter flow cytometry and Attractors to analyze subpopulations based on scatter properties and biochem. processes during **apoptosis** provides a sensitive assay in which to quantitate and characterize the induction of cell death. Depletion of CD4 T cells in vivo by keliximab may reflect, in part, antibody-mediated **apoptosis** of these cells that is dependent on Fc.gamma. receptors.

RE.CNT 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 20 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 135:368870 CA
TI Oncosis is associated with exposure of **phosphatidylserine** residues on the outside layer of the plasma membrane: a reconsideration of the specificity of the annexin V/propidium iodide assay
AU Lecoeur, Herve; Prevost, Marie-Christine; Gougeon, Marie-Lise
CS Unite d'Oncologie Virale, Institut Pasteur, Paris, 75724, Fr.
SO Cytometry (2001), 44(1), 65-72
CODEN: CYTODQ; ISSN: 0196-4763
PB Wiley-Liss, Inc.
DT Journal
LA English
AB Background: Following a lethal injury, two modes of cell death can be distinguished, **apoptosis** and primary necrosis. Cells pass through a pre-lethal stage characterized by a preservation of membrane integrity, in which they shrink (**apoptosis**) or swell (oncosis, the early phase of primary necrosis). During **apoptosis**, a loss of phospholipid asymmetry leads to exposure of **phosphatidylserine** (PS) residues on the outer leaflet of the plasma membrane. We examd. whether the external PS exposure, initially supposed to be specific for **apoptosis**, was also obsd. in oncotic cells. Methods: Human peripheral lymphocytes, Jurkat T cells, U937 cells, or HeLa cells were submitted to either apoptotic or oncotic stimuli. PS external exposure was assessed after binding of FITC-conjugated annexin V as was the loss of membrane integrity after propidium iodide (PI) uptake. Morphol. examn. was performed by optical or electron microscopy. Results: Similarly to apoptotic cells, oncotic cells expose external PS residues while preserving membrane integrity. Consequently, oncotic cells exhibit the annexin V+ PI- phenotype, previously considered to be specific for apoptotic cells. Conclusions: This study concludes that the annexin V/PI assay does not discriminate between **apoptosis** and oncosis and that it can be a useful tool to study oncosis by flow cytometry.

RE.CNT 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 22 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 135:238703 CA
TI Flow cytometry in analysis of cell cycle and **apoptosis**
AU Darzynkiewicz, Zbigniew; Bedner, Elzbieta; Smolewski, Piotr
CS Brander Cancer Research Institute, New York Medical College, Valhalla, NY, 10532, USA
SO Seminars in Hematology (2001), 38(2), 179-193
CODEN: SEHEA3; ISSN: 0037-1963
PB W. B. Saunders Co.
DT Journal; General Review
LA English
AB A review with 71 refs. The capacity for multiparametric measurement of large cell populations rapidly and accurately offered by cytometry has made this methodol. indispensable in studies of cell proliferation and cell death. The reviewed cell cycle applications include (1) the univariate anal. of cellular DNA content for identification of G0/1 vs. S

vs. G2/M cells; (2) discrimination between noncycling (G0; quiescent) and proliferating cells, based on the presence of proliferation-assocd. proteins; (3) identification of mitotic cells by histone H3 phosphorylation; (4) bivariate anal. of expression of cyclins D, E, A, or B1 vs. DNA content; and (5) detection of DNA replicating cells and anal. of cell kinetics from the bivariate distributions of 5-bromo-2'-deoxyuridine (BrdU) incorporation vs. DNA content. For the identification of apoptotic cells and discrimination between **apoptosis** and necrosis, flow cytometry techniques are applied to evaluate for changes in cell morphol., the presence of **phosphatidylserine** on cell surface, collapse of mitochondrial transmembrane potential, DNA fragmentation, and evidence of caspase activation.
RE.CNT 71 THERE ARE 71 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 24 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 135:89338 CA
TI Evaluation of the annexin V method that detects an early change of **apoptosis**
AU Akiyama, Hidehiko; Ino, Teruo; Takasaki, Akihiko; Katsuda, Itsurou; Nagamura, Yoichi; Ezaki, Kohji; Hirano, Masami
CS Dep. Clin. Hematol., Sch. Health Sci., Fujita Health Univ., Toyoake, Aichi, 470-1192, Japan
SO Seibutsu Shiryo Bunseki (2001), 24(2), 121-126
CODEN: SSBUEL; ISSN: 0913-3763
PB Seibutsu Shiryo Bunseki Kagakkai
DT Journal
LA Japanese
AB It has been clear that **apoptosis** plays an important roles in a variety of physiol. and pathol. processes. While a variety of anticancer drugs interact with diverse intracellular mol. targets, they have been shown to induce the common process of **apoptosis** resulting a cell death. **Apoptosis** was characterized by morphol. changes and internucleosomal DNA fragmentation, and accompanied by loss of phospholipid asymmetry across the plasma membrane as well as activation of various proteases. Annexin V can bind specifically to **phosphatidylserine** which becomes accessible on the surface of cells undergoing **apoptosis**. After U937 leukemia cells were incubated with cytarabine (Ara-C), cells were treated with FITC-coupled annexin V and subjected to flow cytometry, detecting cells undergoing **apoptosis**. We compared this method with detection of morphol. changes, DNA fragmentation with electrophoresis, caspase 3 activity and fraction of subdiploid cells with flow cytometry, and demonstrated that annexin V method was useful and ease one for a quant. anal. of cells with **apoptosis**.

L12 ANSWER 25 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 134:248927 CA
TI Various methods of **apoptosis** detection
AU Otsuki, Yoshinori
CS Department of Anatomy and Biology, Osaka Medical College, Osaka, 569-8686, Japan
SO Acta Histochemica et Cytochemica (2000), 33(4), 235-241
CODEN: ACHCBO; ISSN: 0044-5991
PB Japan Society of Histochemistry and Cytochemistry
DT Journal; General Review
LA English
AB A review with 27 refs. **Apoptosis** is cell death defined by some ultrastructural characteristics. DNA agarose gel electrophoresis is suitable for cultured cells consisting of homogeneous cells in which **apoptosis** is relatively easy to induce using appropriate stimuli, but often fails to detect a typical DNA ladder when tissues consisting of heterogeneous cells are used and contain only a few apoptotic cells. It is known that the terminal deoxynucleotidyl transferase (TdT)-mediated

dUTP-biotin nick end-labeling (TUNEL) method detects both apoptotic and necrotic cells, although TUNEL can detect also newly yielded free 3'-OH ends of DNA. Fluorescence dyes specifically bind with DNA, clearly showing fragmented nuclei. Annexin V enables classification of the apoptotic cells into different stages, because it can detect the externalization of **phosphatidylserine** in the cell membrane which occurs at the early stage of **apoptosis**. The disadvantage of fluorescence dyes and annexin V is to be applicable only to unfixed materials. Western blot anal. has several advantages such as its applicability to both cells and tissues, and semiquantification of a protein expressed in materials used, but is unsuitable for anal. of the topog. distribution of cells producing **apoptosis**-related protein such as the caspase family. As mentioned above, most of the **apoptosis** detection methods focus only on one of the apoptotic characteristics, thereby limiting their application to **apoptosis** detection. Therefore, it is required to combine several methods for the precise detection of **apoptosis**.

RE.CNT 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 28 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 134:175084 CA
TI Detection of **apoptosis** by annexin V labeling
AU Bossy-Wetzel, Ella; Green, Douglas R.
CS Division of Cellular Immunology, La Jolla Institute for Allergy and Immunology, San Diego, CA, 92121, USA
SO Methods in Enzymology (2000), 322, 15-18
CODEN: MENZAU; ISSN: 0076-6879
PB Academic Press
DT Journal
LA English
AB A sensitive and rapid method to detect early **apoptosis** is described. The assay is based on the observation that **phosphatidylserine**, a phospholipid normally confined to the cytoplasmic face of the plasma membrane, translocates to the cell surface during **apoptosis** in most cell types and by many apoptotic stimuli. Externalization of PS to the cell surface marks the apoptotic cells to be recognized by neighboring cells or macrophages, facilitating the noninflammatory removal of dying cells by phagocytosis. Once on the cell surface, PS can be detected by binding of fluorescein isothiocyanate (FITC)-labeled annexin V. Annexin V protein belongs to a family of phospholipid-binding proteins that bind specifically, in the presence of calcium, to neg. charged phospholipids such as PS. FITC-labeled annexin V-pos. cells can be detected by flow cytometry or fluorescence microscopy. It is also possible to use annexin V labeling in combination with other dyes, such as propidium iodide and Hoechst 33342, which allows characterization of the progressive stages of **apoptosis**. In most cases, PS translocation to the cell surface occurs before DNA condensation, plasma membrane permeabilization, and membrane blebbing, thus serving as a rapid and convenient measure of early **apoptosis**. (c) 2000 Academic Press.

RE.CNT 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 29 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 134:128021 CA
TI Analysis of apoptotic cells by flow and laser scanning cytometry
AU Darzynkiewicz, Zbigniew; Bedner, Elzbieta
CS Brander Cancer Research Institute, New York Medical College, Valhalla, NY, 10532, USA
SO Methods in Enzymology (2000), 322, 18-39
CODEN: MENZAU; ISSN: 0076-6879
PB Academic Press
DT Journal

LA English
AB A large no. of flow cytometric methods to identify apoptotic cells and analyze morphol., biochem., and mol. changes that occur during **apoptosis** have been developed. These methods are also applicable to the laser scanning cytometer (LSC), a microscope-based cytofluorometer that combines advantages of flow and image cytometry and that, by offering a possibility of assessment of cell morphol., is of particular utility in anal. of **apoptosis**. **Apoptosis**-related changes in cell morphol. assocd. with cell shrinkage and condensation of cytoplasm and chromatin are detected by measurements of the intensity of light scatter of the laser beam in the forward and 90.degree. angle directions. Changes in plasma membrane compn. and function are analyzed by its altered permeability to certain dyes and by the appearance of **phosphatidylserine**, which reacts with annexin V-fluorochrome conjugates on the external surface of the membrane. Decrease in mitochondrial transmembrane potential is measured with several fluorochromes of the rhodamine or carbocyanine family. DNA fragmentation is detected either by measurement of cellular DNA content after elution of the degraded DNA from the cell before or during the staining procedure or by in situ labeling DNA strand breaks. Apoptotic cells are then recognized either on the basis of their reduced DNA-assocd. fluorescence as the cells with fractional DNA content ("sub-G1 cells"), or as the cells with an extensive no. of DNA breaks, resp. Advantages and limitations of the preceding methods are discussed and their adaptation to LSC is presented. (c) 2000 Academic Press.

L12 ANSWER 30 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 134:68239 CA
TI Cell-surface exposure of **phosphatidylserine** correlates with the stage of fludarabine-induced **apoptosis** in chronic lymphocytic leukemia and expression of **apoptosis**-regulating genes
AU Clodi, Katharina; Kliche, Kay Oliver; Zhao, Shourong; Weidner, Douglas; Schenk, Thomas; Consoli, Ugo; Jiang, Shuwei; Snell, Virginia; Andreeff, Michael
CS Department of Molecular Hematology and Therapy, The University of Texas M.D. Anderson Cancer Center, Houston, TX, 77030, USA
SO Cytometry (2000), 40(1), 19-25
CODEN: CYTODQ; ISSN: 0196-4763
PB Wiley-Liss, Inc.
DT Journal
LA English
AB Programmed cell death (PCD) is characterized by a sequence of tightly regulated events that result in the activation of caspases and in internucleosomal DNA cleavage. Late apoptotic events such as DNA-strand breaks can be assayed by in situ end labeling (ISEL) and DNA measurement (sub G1) using flow cytometry. **Phosphatidylserine** (PS) redistribution from the inner plasma membrane leaflet to the outer leaflet, an early event in PCD, can be detected by annexin V (AxV) binding to PS. AxV-fluorescein isothiocyanate (FITC) fluorescence intensity is variable and characterizes different cell populations, denoted here as AxV-neg. (AxVneg), AxV-low-pos. (AxVlo), and AxV-high-pos. (AxVhi). The correlation of 3 methods (ISEL, sub G1 DNA content, and AxV assay) for detecting **apoptosis** was investigated, with focus on differences between populations with different levels of PS. The expression of PCD-regulating Bcl-2 family members in these cell populations was examd. by reverse transcription-polymerase chain reaction (RT-PCR). Chronic lymphocytic leukemia (CLL) cells exposed to fludarabine (FAMP) were used as an in vitro model. Cells with different PS/AxV levels were sepd. using fluorescence-activated cell sorting. Only purified AxVhi cells had high positivity in the ISEL and sub G1 assays (94%, 88.6%, and 98.6%, resp.), indicating that late apoptotic cells are detected equally by all 3 methods. In the AxVlo population, ISEL was pos. in 21% .+-. 13% and DNA sub G1 in 20% .+-. 6.6% of cells, suggesting that AxV identifies early apoptotic cells better than the other assays. Anti-apoptotic Bcl-2 and

Bcl-XL were upregulated by FAMP when cells entered **apoptosis** (AxVlo), as was pro-apoptotic Bcl-XS, which was undetectable in nonapoptotic AxVneg cells. Pro-apoptotic Bax was only expressed in AxVneg and AxVlo cells. Late apoptotic AxVhi cells did not express Bcl-XS or Bax. (1) AxV staining is more sensitive than sub G1 or ISEL in detecting early apoptotic cells; (2) only late apoptotic cells are equally detected by all assays; (3) AxV is a valuable tool in the detection and isolation of apoptotic cells at different stages of PCD; and (4) pro-apoptotic Bcl-XS and Bax are expressed at early, not late, stages of **apoptosis**.

RE.CNT 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 31 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 134:37000 CA
TI Assays for **apoptosis** modulators
IN Elliott, Kathryn J.; Kounnas, Maria Z.; Dyer, Rebecca J.; Munoz, Benito; Wagner, Steven L.; Jones, Jay M.; Corey-naeve, Janis
PA Merck & Co., Inc., USA
SO PCT Int. Appl., 67 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000075160	A1	20001214	WO 2000-US15142	20000601
W: CA, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 2002177120	A1	20021128	US 1999-326472	19990604
EP 1189919	A1	20020327	EP 2000-938044	20000601
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRAI US 1999-326472	A1	19990604		
WO 2000-US15142	W	20000601		

AB Recombinant cells expressing fluorescence resonance energy transfer (FRET) reporter polypeptides and cell-based for **apoptosis**; screening assays for identifying and selecting candidate compds. modulating **apoptosis**.

RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 35 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 133:219806 CA
TI Determination of the chemosensitivity via **phosphatidyl serine** markers
IN Meyer-Almes, Franz Josef
PA Evotec Analytical Systems G.m.b.H., Germany
SO PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000054048	A1	20000914	WO 2000-EP2161	20000311
W: JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
DE 19910955	A1	20000928	DE 1999-19910955	19990312
EP 1161683	A1	20011212	EP 2000-925109	20000311
EP 1161683	B1	20030604		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				

IE, FI
AT 242483 E 20030615 AT 2000-925109 20000311
PRAI DE 1999-19910955 A 19990312
EP 1999-108496 A 19990430
WO 2000-EP2161 W 20000311

AB The invention relates to a method for detg. the chemosensitivity of cells vis-a-vis at least one substance by measuring the level of **apoptosis** induced by the at least one substance. According to the inventive method, the cells are incubated simultaneously with a cytostatic agent and at least one marker whose interaction with **phosphatidyl serine** can be detected and the interaction between the marker and the **phosphatidyl serine** is detected after a certain period of time. Thus blood or bone marrow cells were incubated with the **phosphatidyl serine** marker Annexin V-Alexa 568, BOBO dye, and various antitumor agents, e.g. actinomycin D in a culture medium, contg. calcium. Apoptotic and necrotic cells were quantified based on their different colors via fluorescence microscopy. The method can also be used to det. the effect of environmental toxic substances on cells.

RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 36 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 133:190140 CA
TI Comparison of DiOC6(3) uptake and annexin V labeling for quantification of **apoptosis** in leukemia cells and non-malignant T lymphocytes from children
AU Ozgen, Unsal; Savasan, Sureyya; Buck, Steven; Ravindranath, Yaddanapudi
CS Division of Hematology/Oncology, Barbara Ann Karmanos Cancer Institute, Children's Hospital of Michigan, Wayne State University, Detroit, MI, USA
SO Cytometry (2000), 42(1), 74-78
CODEN: CYTODQ; ISSN: 0196-4763
PB Wiley-Liss, Inc.
DT Journal
LA English

AB Early during **apoptosis**, there is a redn. in mitochondrial transmembrane potential (MTP) and externalization of **phosphatidylserine** (PS) in cell membrane prior to eventual cell death. Flow cytometric detection techniques targeting these changes, redn. of DiOC6(3) uptake upon the collapse of MTP and annexin V binding to PS have been successfully used to detect apoptotic cells. These methods have given comparable results when cell lines were used. We compared the two different techniques, DiOC6(3) uptake and Annexin V-propidium iodide co-labeling in the quantification of cytarabine, vincristine and daunorubicin induced **apoptosis** on three leukemia cell lines (HL-60, CEM, U937), and bone marrow blasts from 26 children with acute myeloid leukemia, 14 with T cell acute lymphoblastic leukemia. Anti-Fas-induced **apoptosis** in culture-grown peripheral blood T lymphocytes on 18 samples from 9 children with non-malignant conditions were also studied by these techniques. Our results showed that there is a correlation ($P < 0.05$) between the **apoptosis** rates measured by these two techniques for drug-induced **apoptosis** in myeloid and lymphoid blasts, and for anti-Fas mAb-induced **apoptosis** in T lymphocytes. This data suggests that redn. of the MTP and PS externalization may be common to many apoptotic pathways and techniques targeting either of these changes may be used in quantification of **apoptosis** in different clin. samples.

RE.CNT 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 37 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 133:133064 CA
TI There is substantial nuclear and cellular disintegration before detectable **phosphatidylserine** exposure during the camptothecin-induced **apoptosis** of HL-60 cells

AU King, Malcolm A.; Radicchi-Mastroianni, Monica A.; Wells, John V.
CS Department of Clinical Immunology, Pacific Laboratory Medical Services, Royal North Shore Hospital, St. Leonards, 2065, Australia
SO Cytometry (2000), 40(1), 10-18
CODEN: CYTODQ; ISSN: 0196-4763
PB Wiley-Liss, Inc.
DT Journal
LA English
AB Background: An early sign of **apoptosis** in many cells is the appearance of **phosphatidylserine** (PS) on the outside of the plasma membrane, while the cells still retain the ability to exclude DNA-binding mols. such as propidium iodide and 7-aminoactinomycin D (7-AAD). The protein annexin V binds preferentially to PS and has often been used to monitor the early phase of **apoptosis**. There have been some conflicting results concerning whether annexin V binds to camptothecin (CAM)-treated HL-60 cells, a commonly used model for **apoptosis**. We investigated the effects of culturing HL-60 cells for up to 8 h with a range of CAM concns. Methods: We used flow cytometry to measure cellular light scatter, annexin V-FITC binding, and 7-AAD uptake, and DNA content after fixation and permeabilization. We also used microscopy to examine the morphol. of cells (both unsorted and sorted according to their light scatter) after cytocentrifugation. Results: We found that CAM caused the rapid appearance of low light scatter apoptotic bodies. Even among cells with "normal" light scatter, there was widespread DNA cleavage and nuclear fragmentation by 3 h. The percentage of apoptotic bodies peaked at about 4 h and it was only afterward that annexin V binding could be detected to both intact cells and to apoptotic bodies. When they first appeared, the intact annexin V+ cells had S-phase DNA content. Conclusions: During CAM-induced **apoptosis** of HL-60 cells, the external exposure of PS can either precede or follow DNA cleavage, which suggests that PS exposure is not always an indicator of early **apoptosis**.
RE.CNT 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 39 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 132:331668 CA
TI Methods for the temporal analysis of programmed cell death in living cells using reagent having affinity for **phosphatidylserine**
IN Maiese, Kenneth; Vincent, Andrea M.
PA Wayne State University, USA
SO U.S., 16 pp., Cont.-in-part of U.S. Ser. No. 144,045.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6063580	A	20000516	US 1999-275831	19990325
	US 5939267	A	19990817	US 1998-144045	19980831
	WO 2000013022	A1	20000309	WO 1999-US19767	19990827
	W: CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	EP 1110087	A1	20010627	EP 1999-968262	19990827
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRAI	US 1998-144045	A2	19980831		
	US 1999-275831	A	19990325		
	WO 1999-US19767	W	19990827		

AB Methods for detg. the induction and assessing the course of programmed cell death (PCD) over time in living cells are provided. The methods of the present invention comprise the steps of contacting viable cells with a detectable reagent having high affinity for **phosphatidylserine**,

qual. and/or quant. detecting the cells that have reacted with the detectable reagent, removing the detectable reagent, recontacting the cells with the detectable reagent and qual. and/or quant. detecting cells that have reacted with the detectable reagent. The methods of the present invention are performed with cells maintained in a viable state, thereby allowing detection of the induction and assessment of the progression of PCD over time. Hippocampal neuronal cultures were treated with sodium nitroprusside, a NO generator, and stained for externalization of **phosphatidylserine** with annexin V conjugated to phycoerythrin. The annexin V was removed in Ca-free conditions. Neurons were examided by microscopy.

RE.CNT 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 41 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 132:262288 CA
TI A novel 96-well scintillation proximity assay for the measurement of **apoptosis**
AU McMurtrey, Amy E.; Graves, Robert J.; Hooley, Jeff; Brophy, Gerard; Phillips, Gail D. Lewis
CS Genentech, Inc., South San Francisco, CA, 94080, USA
SO Cytotechnology (1999), 31(3), 271-282
CODEN: CYTOER; ISSN: 0920-9069
PB Kluwer Academic Publishers
DT Journal
LA English
AB The translocation of phospholipids across the plasma membrane has been widely documented as one of the earliest measurable biochem. events of **apoptosis**. Using fluorescently labeled annexin V, which preferentially binds **phosphatidylserine** (PS) in the presence of Ca2+, the externalization of PS can be measured and **apoptosis** quantified using flow cytometry. Conventional detection methods utilizing annexin V, while faster than in situ DNA end-labeling or DNA laddering, require extensive sample prepn. which may compromise samples and makes rapid, high vol. screening prohibitive. This paper describes a novel assay for the measurement of **apoptosis** based upon binding of radiolabeled annexin V to apoptotic cells attached to the growth surface of a 96-well scintillating microplate (Cytostar-T). We compared measurements of **apoptosis** made by flow cytometry to those obtained with the scintillating microplate in three model systems, treatment of: mouse connective tissue (L-M) cells with lymphotoxin (LT), human lung carcinoma (H460) cells with Apo-2 ligand and human umbilical vein endothelial (HUVE) cells with staurosporine. In this assay, we compare both direct and indirect labeling methods by utilizing either iodinated annexin V or biotinylated annexin V/[35S] streptavidin to radiolabel apoptotic cells. The signal detected is a direct consequence of the binding of annexin V to externalized PS on apoptotic cells and the proximity of the label to the base of the plate. Using this method, sepn. of bound and unbound radiolabel signal occurs directly within the well resulting in a sensitive assay that requires minimal manipulation and can accommodate a large no. of samples.

RE.CNT 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 42 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 132:177746 CA
TI Methods for the temporal analysis of programmed cell death in living cells
IN Maiese, Kenneth; Vincent, Andrea M.
PA Wayne State University, USA
SO PCT Int. Appl., 31 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000013022	A1	20000309	WO 1999-US19767	19990827
	W: CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	US 5939267	A	19990817	US 1998-144045	19980831
	US 6063580	A	20000516	US 1999-275831	19990325
	EP 1110087	A1	20010627	EP 1999-968262	19990827
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRAI	US 1998-144045	A	19980831		
	US 1999-275831	A	19990325		
	WO 1999-US19767	W	19990827		

AB Methods for detg. the induction and assessing the course of programmed cell death (PCD) over time in living cells are provided. The methods of the present invention comprise the steps of contacting viable cells with a detectable reagent having high affinity for **phosphatidylserine**, qual. and/or quant. detecting the cells that have reacted with the detectable reagent, removing the detectable reagent, recontacting the cells with the detectable reagent and qual. and/or quant. detecting cells that have reacted with the detectable reagent. The methods of the present invention are performed with cells maintained in a viable state, thereby allowing detection of the induction and assessment of the progression of PCD over time.

RE.CNT 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 44 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 132:61152 CA
TI Automatic image analysis for quantification of **apoptosis** in animal cell culture by annexin-V affinity assay
AU Plasier, B.; Lloyd, D. R.; Paul, G. C.; Thomas, C. R.; Al-Rubeai, M.
CS SERC Centre for Bioprocess Engineering, School of Chemical Engineering, University of Birmingham, Birmingham, B15 2TT, UK
SO Journal of Immunological Methods (1999), 229(1-2), 81-95
CODEN: JIMMBG; ISSN: 0022-1759
PB Elsevier Science B.V.
DT Journal
LA English
AB **Apoptosis** is a form of cell death in which the dying cell plays an active part in its demise. At the morphol. level, it is characterized by cell shrinkage rather than the swelling seen in necrotic cell death. In cell culture, **apoptosis** limits the yield of economically and medically important products, and can result in synthesis of imperfect mols. Therefore, this process must be identified, monitored and fully understood, so that a means to regulate it can be developed. We have developed a new automatic image anal. assay for detecting **apoptosis** in animal cell culture on the basis of the annexin-V affinity assay. The results of this assay were compared with data generated by flow cytometry and manual scoring. All three methods were found to correspond well but image anal. like flow cytometry offers operator-independent results, and can be used as a tool for rapid monitoring of viable cell no., **apoptosis** and necrosis in animal cell culture. Furthermore, redn. in cell size was measured and was found to precede the appearance of **phosphatidylserine** on the cell surface.

RE.CNT 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 46 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 131:179236 CA
TI Evaluation of cytarabine-induced **apoptosis** in leukemic cell lines. Utility of annexin V method

AU Akiyama, Hidehiko; Suzuki, Kazuhiro; Ino, Teruo; Katsuda, Itsurou; Hirano, Masami
CS Dep. Med., Fujita Health Univ. Sch. Med., Toyoake, 470-1192, Japan
SO Rinsho Byori (1999), 47(8), 774-779
CODEN: RBYOAI; ISSN: 0047-1860
PB Rinsho Byori Gakkai
DT Journal
LA Japanese
AB **Apoptosis** is a morphol. and biochem. distinct form of cell death that occurs under a variety of physiol. and pathol. conditions. Time course of cytarabine-induced **apoptosis** was examd. morphol., using annexin V method, TUNEL method, and fluorometric assay for caspase-3 activity, using leukemic cell lines. Morphol. changes characteristic of **apoptosis** were obsd. in U937 and HL60 cells after 4-h incubation with cytarabine and progressively evident until 48-h incubation, but rarely found in K562 cells. In annexin V method and assay for caspase-3 activity, changes accompanied by **apoptosis** could also be detected at 4-h incubation with cytarabine, but in TUNEL method, they were not found until 24-h incubation. The advantage of annexin V method which detects **phosphatidylserine** emerging on cell surface during the early course of **apoptosis** included simplicity and rapidity of the procedure and short time requirement for **apoptosis** to appear after incubation with cytarabine. Usefulness of annexin V method in a study of clin. samples was discussed.

L12 ANSWER 47 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 131:127386 CA
TI Methods for the temporal analysis of programmed cell death in living cells
IN Maiese, Kenneth; Vincent, Andrea M.
PA Wayne State University, USA
SO U.S., 14 pp.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5939267	A	19990817	US 1998-144045	19980831
	US 6063580	A	20000516	US 1999-275831	19990325
	WO 2000013022	A1	20000309	WO 1999-US19767	19990827
	W: CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	EP 1110087	A1	20010627	EP 1999-968262	19990827
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRAI	US 1998-144045	A2	19980831		
	US 1999-275831	A	19990325		
	WO 1999-US19767	W	19990827		

AB Methods for detg. the induction and assessing the course of programmed cell death (PCD) over time in living cells are provided. The methods of the present invention comprise the steps of maintaining a viable cell on a slide, staining the cell with annexin V to label any **phosphatidylserine** residues which may be present on the cell surface, visualizing the stained cell, removing the stain, sustaining the cell in culture, restaining the cell with annexin V to again label any **phosphatidylserine** residues present on the cell surface and visualizing the stained cell. The methods of the present invention are performed with cells maintained in a viable state, thereby allowing detection of the induction and assessment of the progression of PCD over time.
RE.CNT 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 48 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 131:113270 CA
TI Direct temporal analysis of **apoptosis** induction in living adherent neurons
AU Vincent, Andrea M.; Maiese, Kenneth
CS Laboratory of Cellular and Molecular Cerebral Ischemia, Departments of Neurology and Anatomy and Cell Biology, Center for Molecular and Cellular Toxicology and Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI, 48201, USA
SO Journal of Histochemistry and Cytochemistry (1999), 47(5), 661-671
CODEN: JHCYAS; ISSN: 0022-1554
PB Histochemical Society, Inc.
DT Journal
LA English
AB Destruction of neurons through the genetically directed process of programmed cell death (PCD) is an area of intense interest because this is the underlying mechanism in a variety of developmental and neurodegenerative diseases. The ability to identify and track viable neurons subjected to PCD could be invaluable in development of strategies to prevent or reverse the downstream mechanisms of neuronal PCD. We have developed a novel assay for PCD in viable, adherent cells using annexin V labeling. Annexin V binds to the highly neg. charged plasma membrane **phosphatidylserine** residues that undergo membrane translocation during PCD. Current annexin V techniques are almost exclusively restricted to flow cytometric anal. Our unique technique permits repeated examn. of individual viable neurons without altering their survival. Correlation with electron microscopy and dye exclusion assays demonstrate both sensitivity and specificity for our method to detect PCD. To our knowledge, this is the first account of a technique that pos. identifies PCD in viable, adherent cells.
RE.CNT 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 50 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 130:335014 CA
TI Distinguishing of viable, early apoptotic and necrotic cells
IN Bolton, Wade E.; Koester, Steven K.
PA Coulter International Corp., USA
SO PCT Int. Appl., 21 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9924832	A1	19990520	WO 1998-US23667	19981106
	W: JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	US 5945291	A	19990831	US 1997-966937	19971110
PRAI	US 1997-966937		19971110		

AB The present invention provides a method for distinguishing between viable, early apoptotic, late apoptotic and necrotic cells utilizing multi-color immunofluorescence. The method of the invention involves providing a first binding protein specific for an apoptotic-assocd. antigen labeled with a first visually detectable label, a second binding protein specific for an apoptotic-assocd. antigen labeled with a second visually detectable label, and a third binding protein specific for an intracellular antigen common to eukaryotic cells labeled with third visually detectable label, wherein said first, second, and third visually detectable labels are distinguishable. In a currently preferred embodiment, the invention provides a method involving the steps of contacting a sample of cells with anti-tubulin-FITC, thereby providing pos. and neg. anti-tubulin-FITC populations, contacting the cells with APO2.7-phycoerythrin,

permeabilizing the cells with digitonin, staining the cells with APO2.7-phycoerythrin-cyanin dye 5, and analyzing the cells by flow cytometry to distinguish viable cells, early apoptotic cells, late apoptotic cells and necrotic cells.

RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 51 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 130:308709 CA
TI Annexin V binding assay as a tool to measure **apoptosis** in differentiated neuronal cells
AU Schutte, B.; Nuydens, R.; Geerts, H.; Ramaekers, F.
CS Department of Molecular Cell Biology and Genetics, University of Maastricht, Maastricht, 6200 MD, Neth.
SO Journal of Neuroscience Methods (1998), 86(1), 63-69
CODEN: JNMEDT; ISSN: 0165-0270
PB Elsevier Science B.V.
DT Journal
LA English
AB We describe a rapid and reliable method to quantitate the extent of **apoptosis** in neuronal cell cultures. Based on their annexin V-affinity, resulting from **phosphatidylserine** (PS) exposure at the outer leaflet of the plasma membrane, apoptotic cells can be distinguished from annexin V-neg. living cells, by using microscopic and flow cytometric procedures. When combined with propidium iodide (PI) the double labeling procedure allows a further distinction of necrotic (annexin V+/PI+), apoptotic (annexin V+/PI-) cells. Furthermore, when the cells are incubated with annexin V prior to harvesting, the former cell populations can be sepd. from cells damaged during isolation (annexin V-/PI+). In the present paper, we show that the annexin V-binding assay is also applicable to differentiated neuronal cells with fragile neurite outgrowths.

RE.CNT 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 52 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 130:293614 CA
TI Green fluorescent protein-annexin fusion proteins with useful fluorescence and phospholipid binding properties
IN Ernst, Joel D.
PA The Regents of the University of California, USA
SO PCT Int. Appl., 23 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9919470	A2	19990422	WO 1998-US21444	19981009
	WO 9919470	A3	19990701		
	W: AU, CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	US 6511829	B1	20030128	US 1997-948276	19971009
	AU 9897983	A1	19990503	AU 1998-97983	19981009
	EP 1021465	A2	20000726	EP 1998-952233	19981009
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2001520008	T2	20011030	JP 2000-516023	19981009
PRAI	US 1997-948276	A	19971009		
	WO 1998-US21444	W	19981009		

AB Bifunctional green fluorescent protein (GFP)-annexin fusion proteins combine the inherent strong visible fluorescent properties of GFPs with the anionic phospholipid binding specificity of annexins. Recombinant

host cells, esp. bacteria, are used to efficiently express the fusion proteins in high yield and sol. form, suitable for rapid, one-step affinity purifn. The endogenously fluorescent **phosphatidylserine** -binding proteins contg. Aequorea victoria GFP fused to annexins offer highly sensitive detection of apoptotic cells by flow cytometry or fluorescent microscopy, and offer several advantages to chem. modified annexins. Uses include selective cellular and biochem. labeling, particularly anionic species, such as selectively labeling apoptotic cells.

L12 ANSWER 53 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 130:51395 CA
TI Development of carboxy SNARF-1-AM and annexin V assays for the determination of **apoptosis** in heterogeneous cultures
AU Ishaque, A.; Al-Rubeai, M.
CS Sch. Chem. Eng., Univ. Birmingham, Birmingham, UK
SO New Developments and New Applications in Animal Cell Technology, Proceedings of the ESACT Meeting, 15th, Tours, Fr., Sept. 1997 (1998), Meeting Date 1997, 259-261. Editor(s): Merten, Otto-Wilhelm; Perrin, Pierre; Griffiths, Bryan. Publisher: Kluwer, Dordrecht, Neth. CODEN: 66UJA7
DT Conference
LA English
AB Accurate identification and quantitation of **apoptosis** is essential for developing efficient strategies for optimization of culture survivability and productivity. Flow cytometry in conjunction with several fluoroprobes is increasingly used to identify apoptotic cells. We have examd. the possibility of using carboxy SNARF-1-AM, a pH sensitive fluoroprobe and FITC-labeled annexin V, a probe specific to **phosphatidylserine** exposed on the outer surface of apoptotic cells. Intracellular acidification was shown to precede the occurrence of **apoptosis** thereby proving to be an early indicator of cellular deterioration and cell death. Annexin V in combination with propidium iodide enabled identification of viable, transient apoptotic and necrotic cells in heterogeneous cultures. Metabolic activity (pHi), and cell death population dynamics (viable/apoptotic/necrotic fraction) were therefore effectively and reliably detd. using flow cytometry.
RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 54 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 130:13028 CA
TI A comparative study of different methods for the assessment of **apoptosis** and necrosis in human eosinophils
AU Walsh, Garry M.; Dewson, Grant; Wardlaw, Andrew J.; Levi-Schaffer, Francesca; Moqbel, Redwan
CS Institute of Medical Sciences, Department of Medicine and Therapeutics, University of Aberdeen Medical School, Forester Hill, Aberdeen, AB24 2ZD, UK
SO Journal of Immunological Methods (1998), 217(1-2), 153-163 CODEN: JIMMBG; ISSN: 0022-1759
PB Elsevier Science B.V.
DT Journal
LA English
AB Eosinophils, prominent cells in asthmatic inflammation, undergo **apoptosis** or programmed cell death following deprivation of contact with survival-promoting cytokines such as IL-5 and GM-CSF. The aim of this study was to assess a no. of techniques for the quantification of **apoptosis** in human eosinophils cultured with or without IL-5 or GM-CSF and following staurosporine treatment. The relationship between **apoptosis** and necrosis in eosinophils was also detd. Eosinophils 'aged' in vitro for 48 h exhibited endonuclease DNA degrdn., apoptotic morphol., increased red autofluorescence and externalization of **phosphatidylserine** (PS) as assessed by binding of FITC-labeled

annexin V. Annexin V-FITC binding was first detectable in eosinophils maintained at 37.degree. for 5 h post-purifn. This method proved to be the most sensitive marker of **apoptosis**. Morphol. assessment of wet prepns. of eosinophils by Kimura staining was found to be the next most-sensitive marker followed by increased red autofluorescence. The latter was a relatively insensitive method for the detection of **apoptosis**. At 5, 20 and 24 h of culture trypan blue exclusion indicated that eosinophil viability was high (85-90% viable cells). However, propidium iodide (PI) staining and flow cytometry revealed that, by 24 h, approx. 75% of cells had compromised membrane integrity. Eosinophils maintained in IL-5 or GM-CSF exhibited a non-apoptotic morphol. and levels of annexin V-FITC binding and PI uptake similar to that of freshly isolated cells. Staurosporine (10-5 M) treatment of eosinophils maintained in IL-5 or GM-CSF resulted in significant levels of apoptotic morphol. at 2 h (23.8%.+-.6.9, $p<0.025$) which was assocd. with negligible annexin binding. At 6 h post-staurosporine treatment significant annexin-FITC binding (38%.+-.1.5, $p<0.025$) was obsd. compared with 93%.+-.1.2 of eosinophils displaying apoptotic morphol. Exclusion of PI demonstrated membrane integrity at all time points up to 6 h. Thus, eosinophils aged in vitro in the absence of viability-promoting cytokines exhibit evidence of both **apoptosis** and necrosis simultaneously. In contrast, staurosporine-treated eosinophils exhibited both membrane integrity and rapid **apoptosis**-assocd. morphol. changes detected by single step Kimura staining which preceded externalization of PS.

RE.CNT 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 55 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 129:310554 CA
TI **Apoptosis**-like, reversible changes in plasma membrane asymmetry and permeability, and transient modifications in mitochondrial membrane potential induced by curcumin in rat thymocytes
AU Jaruga, Ewa; Salvioli, Stefano; Dobrucki, Jurek; Chrul, Slawomir; Bandorowicz-Pikula, Joanna; Sikora, Ewa; Franceschi, Claudio; Cossarizza, Andrea; Bartosz, Grzegorz
CS Department of Molecular Biophysics, University of Lodz, Lodz, 90-237, Pol.
SO FEBS Letters (1998), 433(3), 287-293
CODEN: FEBLAL; ISSN: 0014-5793
PB Elsevier Science B.V.
DT Journal
LA English
AB Curcumin (diferuoylmethane) is a natural compd. with anticarcinogenic activities which is able to exert either proapoptotic or antiapoptotic effects in different cell types. This paper focuses on the sequence and extent of primary events induced by curcumin, in comparison with those occurring during dexamethasone-induced **apoptosis** in rat thymocytes. It also presents annexin VI-FITC as a new probe for studying membrane asymmetry. Curcumin readily penetrates into the cytoplasm and is able to accumulate in membranous structures such as plasma membrane, endoplasmic reticulum and nuclear envelope. Curcumin-treated cells exhibit typical features of apoptotic cell death, including shrinkage, transient **phosphatidylserine** exposure, increased membrane permeability and decrease in mitochondrial membrane potential. However, nuclei morphol., DNA fragmentation, the extent and time-course of membrane changes are different from those obsd. during dexamethasone-induced **apoptosis**, suggesting that, despite many similarities, the mode of action and the events triggered by curcumin are different from those occurring during typical **apoptosis**.

RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 57 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 129:158648 CA
TI Analysis of **apoptosis** by flow cytometry

AU Gorczyca, Wojciech; Melamed, Myron R.; Darzynkiewicz, Zbigniew
CS The Cancer Research Institute, New York Medical College, Elmsford, NY, USA
SO Methods in Molecular Biology (Totowa, New Jersey) (1998), 91(Flow Cytometry Protocols), 217-238
CODEN: MMBIED; ISSN: 1064-3745
PB Humana Press Inc.
DT Journal
LA English
AB Methods based on variety of markers are presented along with protocols for the anal. of **apoptosis** and necrosis. The following topics are covered: cell morphol. using light and UV microscopy in combination with staining; gel electrophoresis, flow cytometry, cellular DNA measurements, DNA strand-break labeling assay, detection of apoptic cells by light scattering anal., exclusion of PI combined with uptake of mitochondrial probe rhodamine 123, and detection of **phosphatidylserine** with annexin V-FITC conjugate.
RE.CNT 78 THERE ARE 78 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 58 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 129:146622 CA
TI Preparation and characterization of an endogenously fluorescent annexin for detection of apoptotic cells
AU Ernst, Joel D.; Yang, Lin; Rosales, Jesusa L.; Broaddus, V. Courtney
CS Division of Infectious Diseases and Rosalind Russell Arthritis Research Laboratory, San Francisco General Hospital and University of California, San Francisco, CA, 94143, USA
SO Analytical Biochemistry (1998), 260(1), 18-23
CODEN: ANBCA2; ISSN: 0003-2697
PB Academic Press
DT Journal
LA English
AB Annexin proteins specifically bind anionic phospho-lipids such as **phosphatidylserine**, which are normally confined to the cytoplasmic leaflet of cellular membranes. During programmed cell death, or **apoptosis**, this phospholipid asymmetry is lost, and anionic phospholipids are exposed on the extracellular leaflet of the plasma membrane where they are accessible to exogenously added, labeled annexins. Chem. [e.g., fluoroscein isothiocyanate (FITC)]-modified annexin V has been widely used to detect and enumerate apoptotic cells by flow cytometry. We prepd. chimeric proteins contg. green fluorescent protein (GFP) fused to annexin V. A chimera contg. GFP fused to the C-terminus of annexin V was sol. and fluorescent, but was unable to bind phospholipids. In contrast, a chimera contg. GFP fused to the N-terminus of annexin V specifically bound apoptotic cells. GFP-annexin V represents a sensitive and facile alternative to FITC-annexin V for studies of **apoptosis** . (c) 1998 Academic Press.
RE.CNT 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 59 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 129:92427 CA
TI Early features of **apoptosis** detected by four different flow cytometry assays
AU Overbeeke, R.; Steffens-Nakken, H.; Vermes, I.; Reutelingsperger, C.; Haanen, C.
CS Hospital Group, Department of Clinical Chemistry, Medical Spectrum Twente, Enschede, 7500 KA, Neth.
SO Apoptosis (1998), 3(2), 115-121
CODEN: APOPFN; ISSN: 1360-8185
PB Rapid Science Ltd.
DT Journal
LA English
AB The objective of this study was to investigate the sensitivity,

specificity and reproducibility of some frequently used **apoptosis** assays. The degree of **apoptosis** was tested in two T-lymphoblastoid cell lines, HSB and Jurkat, in which **apoptosis** was induced by ionizing radiation. HSB and Jurkat samples were taken before, and 0, 2, 4, 6, 8 and 24 h after irradn. with 6 and 10 Gy, or with 10 and 14 Gy, resp. Four frequently used flow cytometric techniques were evaluated: (i) Annexin V/Propidium Iodide assay, detecting the translocation of **phosphatidylserine** to the outer leaflet of the plasma membrane, simultaneously with preservation of the membrane integrity; (ii) Terminal deoxynucleotidyl Transferase (TdT) Uridine triphosphate (UTP) nick end labeling (TUNEL), revealing the presence of DNA strand breaks; (iii) DNA-flow cytometry, measuring DNA-stainability (DNA-fragmentation assay) and (i.v.) Phycoerythrin-labeled (PE) Apo2.7-assay, a monoclonal antibody against 7A6 antigen, a protein, which becomes exposed upon the mitochondrial membrane during **apoptosis** . As a general std. for identifying that **apoptosis** had occurred, the cells were assessed for the presence of DNA-laddering on agar gel electrophoresis and by demonstration of characteristic cell morphol. Results were as follows: Fluorescein Isothiocyanate (FITC)-labeled Annexin V/Propidium iodide flow cytometry appeared to be the most sensitive, the most specific and the most user-friendly test for measurement of **apoptosis** of cells in culture conditions in suspension. The expression of 7A6 antigen on the mitochondrial membrane appeared to be not specific for apoptotic cell death.

RE.CNT 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 63 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 128:10306 CA
TI Method of isolating regulators of T-cell activation
IN Lin, Augustine Y.-T.; Umlauf, Scott W.; Batzer, Andreas G.
PA T Cell Sciences, Inc., USA; Lin, Augustine Y.-T.; Umlauf, Scott W.; Batzer, Andreas G.
SO PCT Int. Appl., 86 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9739722	A2	19971030	WO 1997-US7052	19970425
	W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	AU 9728148	A1	19971112	AU 1997-28148	19970425
PRAI	US 1996-639286		19960425		
	WO 1997-US7052		19970425		

AB This invention provides a multi-step, high-throughput primary screen to identify immune regulators of T-cell activation for use as therapeutic agents. Compns. or compds. are screened for their ability to stimulate or inhibit the expression of a reporter gene operatively linked to specific transcriptional control sequences in T cells. A compn. or compd. identified as an immune stimulator or inhibitor by the primary screen of this invention can then be further characterized to det. the target mol. on which the compn. or compd. acts to regulate T-cell activation and T-cell activation-dependent processes such as **apoptosis**.

L12 ANSWER 64 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 127:290007 CA

TI Early detection of **apoptosis** using a fluorescent conjugate of annexin V
AU Zhang, Guohong; Gurtu, Vanessa; Kain, Steven R.; Yan, Guochen
CS CLONTECH Lab., Palo Alto, CA, 94303, USA
SO BioTechniques (1997), 23(3), 525-526, 528-531
CODEN: BTNQDO; ISSN: 0736-6205
PB Eaton
DT Journal
LA English
AB **Apoptosis** of mammalian cells is accompanied by various morphol. changes including nuclear condensation, DNA fragmentation and cell surface changes. Methods developed over the past few years have focused on detection of DNA-assocd. changes that occur rather late in **apoptosis**. However, detection of **apoptosis** at early stages, before gross morphol. changes, is crit. for understanding the pathways of programmed cell death. In this report, we describe a rapid and reliable assay for detecting early stages of **apoptosis**. This assay is based on the observation that soon after initiating **apoptosis**, most mammalian cell types translocate **phosphatidylserine** (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS can be specifically detected by staining with fluorescein isothiocyanate (FITC)-labeled annexin V (annexin V-FITC), a protein with a strong, natural affinity for PS. Using this assay, we have detected apoptotic cells in culture, in real time, using fluorescence microscopy and flow cytometry. In combination with vital dye staining, the progressive stages of **apoptosis** were obsd. PS redistribution occurs earlier than DNA-assocd. changes and membrane leakage. In addn., PS externalization occurs during **apoptosis** induced by a variety of stimuli. Therefore, the annexin V binding assay provides an excellent indicator for the early stages of **apoptosis**.

L12 ANSWER 67 OF 67 CA COPYRIGHT 2003 ACS on STN
AN 121:225520 CA
TI Annexin V for flow cytometric detection of **phosphatidylserine** expression on B cells undergoing **apoptosis**
AU Koopman, G.; Reutelingsperger, C. P. M.; Kuijten, G. A. M.; Keehnen, R. M. J.; Pals, S. T.; van Oers, M. H. J.
CS Department of Pathology, Academic Medical Center, Amsterdam, 1105 AZ, Neth.
SO Blood (1994), 84(5), 1415-20
CODEN: BLOOAW; ISSN: 0006-4971
DT Journal
LA English
AB **Apoptosis**, or programmed cell death, is a general mechanism for removal of unwanted cells from the immune system. It is characterized by chromatin condensation, a redn. in cell vol., and endonuclease cleavage of DNA into oligonucleosomal length fragments. **Apoptosis** is also accompanied by a loss of membrane phospholipid asymmetry, resulting in the exposure of **phosphatidylserine** at the surface of the cell. Expression of **phosphatidylserine** at the cell surface plays an important role in the recognition and removal of apoptotic cells by macrophages. Here the authors describe a new method for the detection of apoptotic cells by flow cytometry, using the binding of fluorescein isothiocyanate-labeled annexin V to **phosphatidylserine**. When Burkitt lymphoma cell lines and freshly isolated germinal center B cells are cultured under **apoptosis**-inducing conditions, all cells showing chromatin condensation strongly stain with annexin V, whereas normal cells are annexin V neg. Moreover, DNA fragmentation is only found in the annexin V-pos. cells. The nonvital dye ethidium bromide was found to stain a subpopulation of the annexin V-pos. apoptotic cells, increasing with time. The results indicate that the phase in **apoptosis** that is characterized by chromatin condensation coincides with **phosphatidylserine** exposure. Importantly, it precedes membrane

damage that might lead to release from the cells of enzymes that are harmful to the surrounding tissues. Annexin V may prove important in further unravelling the regulation of **apoptosis**.

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L9 ANSWER 18 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:138065 BIOSIS
DN PREV200100138065
TI Zinc induces mixed types of cell death, necrosis, and **apoptosis**, in Molt-4 cells.
AU Hamatake, Michiko; Iguchi, Kazuhiro; Hirano, Kazuyuki; Ishida, Ryoji (1)
CS (1) Division of Molecular Medicine, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya, 464-8681: rishida@aichigw.aichi-cc.pref.aichi.jp Japan
SO Journal of Biochemistry (Tokyo), (Dec., 2000) Vol. 128, No. 6, pp. 933-939. print.
ISSN: 0021-924X.
DT Article
LA English
SL English
AB To investigate the mode of zinc-induced cell death, the associated morphological changes, and biological events were examined in zinc-treated Molt-4 cells. Fluorescence microscope observations with double staining of zinc-treated cells with Hoechst 33342 and propidium iodide (PI) indicated that the metal induced both necrosis and **apoptosis**. To confirm this, cells were stained with both PI and FITC-labeled **annexin** V, which binds **phosphatidylserine**, and then analyzed by flow cytometry. The results also confirmed that zinc induces mixed types of cell death, necrosis and **apoptosis**, and that the former induction occurs earlier and at a greater frequency. Hallmarks of **apoptosis** such as abnormal chromosome condensation and release of cytochrome c, as well as the appearance of **annexin**-positive cells, appeared along with the expression of mitochondrial membrane protein 7A6. However, zinc did not induce increases in caspase-3 like protease and caspase-8 activities, and caused slightly hypodiploid cells. Furthermore, the induction of cell death and **annexin**-positive cells was not blocked by the caspase inhibitors Ac-YVAD-CHO and Ac-DEVD-CHO. These results indicate that zinc induces both necrosis and **apoptosis**, without caspase-3 activation.

L9 ANSWER 19 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:124358 BIOSIS
DN PREV200100124358
TI Potent induction of **apoptosis** by beta-lapachone in human multiple myeloma cell lines and patient cells.
AU Li, Youzhi (1); Li, Chiang J.; Yu, Donghui; Pardee, Arthur B.
CS (1) Division of Cancer Biology, Dana-Farber Cancer Institute, and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 44 Binney St., Boston, MA, 02115: Youzhi_Li@dfci.harvard.edu USA
SO Molecular Medicine (New York), (December, 2000) Vol. 6, No. 12, pp. 1008-1015. print.
ISSN: 1076-1551.
DT Article
LA English
SL English
AB Background: Human multiple myeloma (MM) remains an incurable hematological malignancy. We have reported that beta-lapachone, a pure compound derived from a plant, can induce cell death in a variety of human carcinoma cells, including ovary, colon, lung, prostate, pancreas, and breast, suggesting a wide spectrum of anticancer activity. Materials and Methods: We first studied anti-survival effects of beta-lapachone in human MM cells by colony formation assay. To determine whether the differential inhibition of colony formation occurs through antiproliferative activity, we performed MTT assays. The cytotoxicity of beta-lapachone on human peripheral blood mononuclear cells was also measured by MTT assay. To determine whether the cell death induced by beta-lapachone occurs through necrosis or **apoptosis**, we used the propidium iodide staining

procedure to determine the sub-G1 fraction, **Annexin**-V staining for externalization of **phosphatidylserine**, and fragmentation of cellular genomic DNA subjected to gel electrophoresis. To investigate the mechanism of anti-MM activity, we examined Bcl-2 expression, cytochrome C release, and poly (ADP ribose) polymerase cleavage by Western blot assay. Results: We found that beta-lapachone (less than 4 muM) inhibits cell survival and proliferation by triggering cell death with characteristics of **apoptosis** in ARH-77, HS Sultan, and MM.1S cell lines, in freshly derived patient MM cells (MM.As), MM cell lines resistant to dexamethasone (MM.1R), doxorubicin (DOX.40), mitoxantrone (MR.20), and mephalan (LR5). Importantly, after treatment with beta-lapachone, we observed no **apoptosis** in peripheral blood mononuclear cells in either quiescent or proliferative states, freshly isolated from healthy donors. In beta-lapachone treated ARH-77, cytochrome C was released from mitochondria to cytosol, and poly (ADP ribose) polymerase was cleaved, signature events of **apoptosis**. Finally, the **apoptosis** induced by beta-lapachone in MM cells was not blocked by either interleukin-6 or Bcl-2, which confer multidrug resistance in MM. Conclusions: Our results suggest potential therapeutic application of beta-lapachone against MM, particularly to overcome drug resistance in relapsed patients.

L9 ANSWER 20 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:123797 BIOSIS
DN PREV200100123797
TI Abrin-a-induced cytotoxicity and **apoptosis** against human leukemic cell lines.
AU Moriwaki, S. (1); Ohba, H.; Nakamura, O.; Sumi, T.; Park, S.; Yasuda, S.; Yamasaki, N. (1); Suzuki, M.; Tsubouchi, H.
CS (1) 6-10-1 Hakozaki, Fukuoka, Fukuoka, 812-0053 Japan
SO Biochemical Society Transactions, (October, 2000) Vol. 28, No. 5, pp. A379. print.
Meeting Info.: 18th International Congress of Biochemistry and Molecular Biology Birmingham, UK July 16-20, 2000
ISSN: 0300-5127.
DT Conference
LA English
SL English

L9 ANSWER 26 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:50370 BIOSIS
DN PREV200100050370
TI Various methods of **apoptosis** detection.
AU Otsuki, Yoshinori (1)
CS (1) Department of Anatomy and Biology, Osaka Medical College, 2-7, Daigaku-machi, Takatsuki, Osaka, 569-8686 Japan
SO Acta Histochemica et Cytochemica, (2000) Vol. 33, No. 4, pp. 235-241. print.
ISSN: 0044-5991.
DT General Review
LA English
SL English
AB **Apoptosis** is cell death defined by some ultrastructural characteristics. DNA agarose gel electrophoresis is suitable for cultured cells consisting of homogeneous cells in which **apoptosis** is relatively easy to induce using appropriate stimuli, but often fails to detect a typical DNA ladder when tissues consisting of heterogeneous cells are used and contain only a few apoptotic cells. It is known that the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labeling (TUNEL) method detects both apoptotic and necrotic cells, although TUNEL can detect also newly yielded free 3'-OH ends of DNA. Fluorescence dyes specifically bind with DNA, clearly showing fragmented nuclei. **Annexin** V enables classification of the apoptotic cells into different stages, because it can detect the externalization of

phosphatidylserine in the cell membrane which occurs at the early stage of **apoptosis**. The disadvantage of fluorescence dyes and **annexin** V is to be applicable only to unfixed materials. Western blot analysis has several advantages such as its applicability to both cells and tissues, and semiquantification of a protein expressed in materials used, but is unsuitable for analysis of the topographic distribution of cells producing **apoptosis**-related protein such as the caspase family. As mentioned above, most of the **apoptosis** detection methods focus only on one of the apoptotic characteristics, thereby limiting their application to **apoptosis** detection. Therefore, it is required to combine several methods for the precise detection of **apoptosis**.

L9 ANSWER 29 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:36869 BIOSIS
DN PREV200100036869
TI Ozone-induced apoptotic response in human bronchial epithelial cells.
AU Nardini, M. (1); Reddy, S. (1); Lavrentiadou, S. N. (1); Tarkington, B. (1); Goldkorn, T. (1); van der Vliet, A. (1); Cross, C. E. (1)
CS (1) Center for Comparative Respiratory Biology and Medicine, UC Davis, Davis, CA USA
SO Free Radical Biology & Medicine, (2000) Vol. 29, No. Supplement 1, pp. S59. print.
Meeting Info.: 7th Annual Meeting of the Oxygen Society San Diego, CA, USA November 16-20, 2000
ISSN: 0891-5849.
DT Conference
LA English
SL English

L9 ANSWER 36 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:492058 BIOSIS
DN PREV200000492179
TI Effect of auranofin, an antirheumatic drug, on neutrophil **apoptosis**.
AU Liu, J.; Akahoshi, T. (1); Namai, R.; Matsui, T.; Kondo, H.
CS (1) Department of Internal Medicine, Kitasato University School of Medicine, Kitasato 1-15-1, Sagamihara, Kanagawa, 228-8555 Japan
SO Inflammation Research, (September, 2000) Vol. 49, No. 9, pp. 445-451. print.
ISSN: 1023-3830.
DT Article
LA English
SL English
AB Objective: The effects of auranofin (AF) on **apoptosis** and on the biological functions of neutrophils were investigated. Methods: Neutrophils were incubated with various concentrations of AF for different periods. Cell viability was determined by the MTS assay and **apoptosis** was evaluated by flow cytometric analysis of propidium iodide (PI)- staining of the nuclei and **annexin**-V staining of **phosphatidylserine** in the cell membrane. The effect of AF on the expression of adhesion molecules (CD62L and CD11b/CD18) and on the generation of O2- by neutrophils was also determined. Results: At a low concentration (1 muM), AF significantly prolonged neutrophil survival by delaying spontaneous **apoptosis**. Neutrophils incubated with AF for 12 and 24 hours maintained the capacity to express adhesion molecules and generate O2-. In contrast, a higher AF concentration (5 muM) shortened neutrophil survival by the induction of cell necrosis. Conclusion: Although the biological significance of inhibitory effect of AF on neutrophil **apoptosis** remains unclear, it seems to be unlikely that AF exerts the anti-inflammatory effect in vivo by directly suppressing neutrophil functions. Since AF has a wide range of effects on leukocytes, its therapeutic benefit in rheumatoid arthritis may be mediated in a complex manner.

L9 ANSWER 37 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:484833 BIOSIS
DN PREV200000484833
TI Tilmicosin induces **apoptosis** in bovine peripheral neutrophils in the presence or in the absence of Pasteurella haemolytica and promotes neutrophil phagocytosis by macrophages.
AU Chin, Alex C.; Lee, Wilson D.; Murrin, Katherine A.; Morck, Douglas W.; Merrill, John K.; Dick, Paul; Buret, Andre G. (1)
CS (1) Department of Biological Sciences, University of Calgary, 2500 University Dr. N.W., Calgary, AB, T2N 1N4 Canada
SO Antimicrobial Agents and Chemotherapy, (September, 2000) Vol. 44, No. 9, pp. 2465-2470. print.
ISSN: 0066-4804.
DT Article
LA English
SL English
AB Pathogen virulence factors and inflammation are responsible for tissue injury associated with respiratory failure in bacterial pneumonia, as seen in the bovine lung infected with Pasteurella haemolytica. Tilmicosin is a macrolide antibiotic used for the treatment of bovine bacterial pneumonia. Recent evidence suggests that tilmicosin-induced neutrophil **apoptosis** may have anti-inflammatory effects. Using bovine leukocytes, we sought to define whether live P. haemolytica affected tilmicosin-induced neutrophil **apoptosis**, assessed the proapoptotic effects of tilmicosin in comparison with other drugs, and characterized its impact on phagocytic uptake of neutrophils by macrophages. Induction of **apoptosis** in the presence or absence of P. haemolytica was assessed by using an enzyme-linked immunosorbent assay for apoptotic nucleosomes. In addition, fluorescent **annexin** -V staining identified externalized **phosphatidylserine** in neutrophils treated with tilmicosin, penicillin, ceftiofur, oxytetracycline; or dexamethasone. Neutrophil membrane integrity was assessed by using propidium iodide and trypan blue exclusion. As phagocytic clearance of apoptotic neutrophils by macrophages contributes to the resolution of inflammation, phagocytosis of tilmicosin-treated neutrophils by esterase-positive cultured bovine macrophages was assessed with light microscopy and transmission electron microscopy. Unlike bovine neutrophils treated with penicillin, ceftiofur, oxytetracycline, or dexamethasone, neutrophils exposed to tilmicosin became apoptotic, regardless of the presence or absence of P. haemolytica. Tilmicosin-treated apoptotic neutrophils were phagocytosed at a significantly greater rate by bovine macrophages than were control neutrophils. In conclusion, tilmicosin-induced neutrophil **apoptosis** occurs regardless of the presence or absence of live P. haemolytica, exhibits at least some degree of drug specificity, and promotes phagocytic clearance of the dying inflammatory cells.

L9 ANSWER 38 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:479053 BIOSIS
DN PREV200000479053
TI **Phosphatidylserine** externalization in human trophoblast differentiation and **apoptosis**.
AU Xu, Bo (1); Lin, Lin (1); Rote, Neal (1)
CS (1) Department of Microbiology and Immunology, Wright State University, Dayton, OH, 45435 USA
SO Journal of Autoimmunity, (Sept., 2000) Vol. 15, No. 2, pp. A61. print.
Meeting Info.: 9th International Symposium on Antiphospholipid Antibodies Tours, France September 12-16, 2000
ISSN: 0896-8411.
DT Conference
LA English
SL English

L9 ANSWER 41 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:455779 BIOSIS
DN PREV200000455779
TI Evidence for **apoptosis** of the majority of T cells activated in vitro with Actinobacillus actinomycetemcomitans.
AU Nalbant, A.; Zadeh, H. H. (1)
CS (1) Immune Response Laboratory, Department of Periodontology, School of Dentistry, University of Southern California, 925 West 34th Street, Los Angeles, CA, 90089 USA
SO Oral Microbiology and Immunology, (October, 2000) Vol. 15, No. 5, pp. 290-298. print.
ISSN: 0902-0055.
DT Article
LA English
SL English
AB Our previous studies had demonstrated that nearly half of all T cells stimulated with Actinobacillus actinomycetemcomitans are activated within a few hours. However, it was not known whether all of these T cells survive. The aim of the present study was to determine whether the T cells activated in response to A. actinomycetemcomitans undergo **apoptosis**. To that end, peripheral blood mononuclear cells were cultured at different time points in the presence of A. actinomycetemcomitans. Flow cytometric analysis demonstrated that, following exposure to a preparation of A. actinomycetemcomitans, T cells progressively externalized their plasma membrane **phosphatidylserine**, as measured by **annexin** V binding. Approximately half of all T cells bound **annexin** V by 96 h. During this period, **Annexin** V-positive T cells also incorporated propidium iodide suggesting loss of membrane integrity. The externalization of **phosphatidylserine** occurred at a higher rate among activated (CD69+) T cells, where roughly two-thirds became **Annexin** V-positive. Flow cytometric analysis also demonstrated shrinkage of the **Annexin** V-positive and propidium iodide-positive T cells. The data presented here provides evidence for the induction of **apoptosis** among the majority of the T cells responding to A. actinomycetemcomitans.

L9 ANSWER 45 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:424575 BIOSIS
DN PREV200000424575
TI Analysis of apoptotic cells by flow and laser scanning cytometry.
AU Darzynkiewicz, Zbigniew (1); Bedner, Elzbieta
CS (1) Brander Cancer Research Institute, New York Medical College, Valhalla, NY, 10532 USA
SO Reed, John C.. Methods in Enzymology, (2000) Vol. 322, pp. 18-39. Methods in Enzymology; Apoptosis. print.
Publisher: Academic Press Inc. 525 B Street, Suite 1900, San Diego, CA, 92101-4495, USA.
ISSN: 0076-6879. ISBN: 0-12-182223-0 (cloth).
DT Book
LA English
SL English

L9 ANSWER 46 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:424574 BIOSIS
DN PREV200000424574
TI Detection of **apoptosis** by **annexin** V labeling.
AU Bossy-Wetzel, Ella (1); Green, Douglas R.
CS (1) Division of Cellular Immunology, La Jolla Institute for Allergy and Immunology, San Diego, CA, 92121 USA
SO Reed, John C.. Methods in Enzymology, (2000) Vol. 322, pp. 15-18. Methods in Enzymology; Apoptosis. print.
Publisher: Academic Press Inc. 525 B Street, Suite 1900, San Diego, CA, 92101-4495, USA.

ISSN: 0076-6879. ISBN: 0-12-182223-0 (cloth).
DT Book
LA English
SL English

L9 ANSWER 48 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:415210 BIOSIS
DN PREV200000415210
TI Caspase and proteasome activity during staurosporin-induced **apoptosis** in lens epithelial cells.
AU Andersson, Madeleine (1); Sjostrand, Johan; Petersen, Anne; Honarvar, Antovan K. S.; Karlsson, Jan-Olof
CS (1) Institute of Anatomy and Cell Biology, Goteborg University, Medicinaregatan 5, SE-405 30, Goteborg Sweden
SO IOVS, (August, 2000) Vol. 41, No. 9, pp. 2623-2632. print.
DT Article
LA English
SL English
AB Purpose. To determine what caspases are activated during staurosporin-induced **apoptosis** in cultured bovine lens epithelial cells (BLECs), to study the time course of caspase activation in relation to morphologic changes, and to investigate the effect of caspase and/or proteasome inhibition on **apoptosis**. Methods. BLECs were incubated with staurosporin at different concentrations or for different times. **Phosphatidylserine** (PS) externalization was detected by **annexin**-V labeling, nuclear morphology was studied by staining with Hoechst 33342 stain (Hoechst, Frankfurt, Germany), and the percentage of apoptotic cells was determined by the TdT-dUTP terminal nick-end labeling (TUNEL) assay. The activity of caspase-1, -2, -3, -4, -8, and -9 as well as the chymotrypsin-like activity of the proteasome was measured by the use of fluorogenic peptide substrates. Inhibition of the proteasome was performed by incubation with 10 muM lactacystin, and caspases were inhibited by 1 muM Z-DEVD-FMK or 20 muM Z-VAD-FMK. Results. Staurosporin treatment caused a dose- and time-dependent increase in the number of apoptotic cells and in caspase-3 activity. Activation of caspase-2, -4, -8, and -9, was also seen. Caspase activity was increased after 3 hours' incubation with 1 muM staurosporin, which is also the time when most cells became **annexin**-V-positive. Nuclear changes indicative of **apoptosis**, viewed with both Hoechst and TUNEL staining, appeared after 4 to 6 hours of staurosporin incubation. Incubation of BLECs with lactacystin caused reduction of proteasome activity and increased **apoptosis**, evidenced in both the TUNEL assay and caspase-3 activation. Preincubation of lens epithelial cells with caspase inhibitors caused complete inhibition of lactacystin- or staurosporin-induced caspase-3 activation (Z-DEVD-FMK/Z-VAD-FMK) and also of caspase-2, -4, -8, and -9 (Z-VAD-FMK), but the reduction in TUNEL-positive cells was only partial. PS translocation and DNA fragmentation after staurosporin treatment occurred despite complete caspase blockade. Conclusions. Staurosporin-induced **apoptosis** in BLECs involves activation of several caspases. Inhibition of the proteasome causes caspase-3 activation and **apoptosis**. Both staurosporin- and lactacystin-induced **apoptosis** can be executed in a caspase-independent manner. The present data are useful for understanding of proteolytic mechanisms during **apoptosis** in lens epithelial cells, which may be an important event in normal lens development as well as in some types of cataract.

L9 ANSWER 51 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:396625 BIOSIS
DN PREV200000396625
TI Induction of oxidative stress and **apoptosis** in myeloma cells by the aziridine-containing agent imexon.
AU Dvorakova, Katerina; Payne, Claire M.; Tome, Margaret E.; Briehl, Margaret M.; McClure, Thomas; Dorr, Robert T. (1)

CS (1) Arizona Cancer Center, 1515 North Campbell Avenue, Tucson, AZ, 85724 USA
SO Biochemical Pharmacology, (15 September, 2000) Vol. 60, No. 6, pp. 749-758. print.
ISSN: 0006-2952.
DT Article
LA English
SL English
AB Imexon is an iminopyrrolidone derivative that has selective antitumor activity in multiple myeloma. The exact mechanism of imexon action is unknown. In human 8226 myeloma cells, the cytotoxicity of imexon was schedule-dependent, and long exposures (gtoreq48 hr) to low concentrations of imexon were most effective at inducing cytotoxicity. Our data suggest that imexon does not affect DNA, but it can alkylate thiols by binding to the sulfhydryl group. We have also demonstrated by HPLC studies that in human 8226 myeloma cells, imexon depletes cellular stores of cysteine and glutathione. Oxidative stress in 8226 cells exposed to imexon was detected by immunohistochemical staining with a monoclonal antibody to 8-hydroxydeoxyguanosine (8-OHdG), followed by confocal microscopy. These images showed increased levels of 8-OHdG in the cytoplasm of cells treated with different concentrations of imexon at 8, 16, and 48 hr. Interestingly, 8-OHdG staining was not observed in the nuclei of imexon-treated cells, in contrast to the diffuse staining seen with t-butyl hydroperoxide. Myeloma cells exposed to imexon showed classic morphologic features of **apoptosis** upon electron microscopy, and increased levels of **phosphatidylserine** exposure, detected as **Annexin**-V binding, on the cell surface. To prevent depletion of thiols, 8226 myeloma cells exposed to imexon were treated with N-acetylcysteine (NAC). Simultaneous, as well as sequential, treatment with NAC before imexon exposure resulted in protection of myeloma cells against imexon-induced cytotoxicity. Conversely, the glutathione synthesis inhibitor buthionine sulfoximine increased imexon cytotoxicity. These data suggest that imexon perturbs cellular thiols and induces oxidative stress leading to **apoptosis** in human myeloma cells.

L9 ANSWER 53 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS·INC. on STN
AN 2000:374735 BIOSIS
DN PREV200000374735
TI Lysophosphatidylcholine induces apoptotic and non-apoptotic death in vascular smooth muscle cells: In comparison with oxidized LDL.
AU Hsieh, Chien-Cheng; Yen, Mao-Hsiung; Liu, Hwan-Wun; Lau, Ying-Tung (1)
CS (1) Department of Physiology, Chang Gung University College of Medicine, 259 Wen Hwa 1Rd., Kwei-Shan, Tao-Yuan Taiwan
SO Atherosclerosis, (August, 2000) Vol. 151, No. 2, pp. 481-491. print.
ISSN: 0021-9150.
DT Article
LA English
SL English
AB Oxidized low-density lipoprotein (oxLDL) plays a key role in the development of atherogenesis, partly by causing injury to vascular cells. However, different preparations of LDL, methods of oxidation, and/or active components often produce cellular effects of various degrees. To explore the quantitative relationship between dose and level of oxidation of the oxLDL utilized, we employed combinations of different levels of oxidation and concentrations of oxLDL to induce cell death in cultured vascular smooth muscle cells (VSMC). We also examined the effect of lysophosphatidylcholine (lysoPC), a putative active component of oxLDL, on VSMCs by determining, in parallel with a cytotoxicity test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay), DNA fragmentation ((3H)thymidine release), and flow cytometric analyses. We found that oxLDL caused cytotoxicity in an oxidative level- and dose-dependent manner, lysoPC also caused dose-dependent cytotoxicity with or without serum. Fragmentation of DNA was observed in both oxLDL- and lysoPC-treated VSMCs. Furthermore, lysoPC-induced DNA ladder was also

demonstrated by gel electrophoresis at a concentration of 25 mumol/l or higher. Flow cytometric analysis yielded similar results for oxLDL- and lysoPC-treated VSMC; namely, an accumulation in the fraction of cells in G0/G1 phase with a reciprocal change in S-phase fraction. Membrane **phosphatidylserine** exposure, detected by **annexin** V staining, provided additional evidence that lysoPC induced significant **apoptosis** in VSMC. Taken together, the degree of oxLDL-induced cytotoxicity/**apoptosis** of VSMC depended on combined effects of oxLDL concentration and oxidative level. Moreover, lysoPC also elicited a dose-dependent **apoptosis** in addition to cytotoxicity.

L9 ANSWER 54 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:369958 BIOSIS
DN PREV200000369958
TI Early detection of **apoptosis** with **annexin** V-enhanced green fluorescent protein.
AU Kain, Steven R. (1); Ma, Jing-Tyan
CS (1) CLONTECH Laboratories, Inc., Palo Alto, CA, 94303-4230 USA
SO Conn, P. Michael. Methods in Enzymology, (1999) Vol. 302, pp. 38-43. Methods in Enzymology; Green fluorescent protein. print. Publisher: Academic Press Inc. 525 B Street, Suite 1900, San Diego, CA, 92101-4495, USA. ISSN: 0076-6879. ISBN: 0-12-182203-6 (cloth).
DT Book
LA English
SL English

L9 ANSWER 59 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:324789 BIOSIS
DN PREV200000324789
TI A carboxy-terminally truncated form of the human immunodeficiency virus type 1 Vpr protein induces **apoptosis** via G1 cell cycle arrest.
AU Nishizawa, Masako; Kamata, Masakazu; Katsumata, Ryoichi; Aida, Yoko (1)
CS (1) Tsukuba Life Science Center, Institute of Physical and Chemical Research (RIKEN), 3-1-1 Koyadai, Tsukuba, Ibaraki, 305-0074 Japan
SO Journal of Virology, (July, 2000) Vol. 74, No. 13, pp. 6058-6067. print. ISSN: 0022-538X.
DT Article
LA English
SL English
AB Viral protein R (Vpr) of human immunodeficiency virus type 1 inhibits cell proliferation by arresting the cell cycle at the G2 phase and inducing to **apoptosis** after G2 arrest. We have reported previously that C81, a carboxy-terminally truncated form of Vpr, interferes with cell proliferation via a novel pathway that is distinct from G2 arrest. However, the mechanism of this effect of C81 is unknown. We demonstrate here that C81 can induce **apoptosis** via G1 arrest of the cell cycle. Immunostaining for various markers of stages of the cell cycle and flow cytometry analysis of DNA content showed that most HeLa cells that had been transiently transfected with a C81 expression vector were arrested at the G1 phase and not at the G2 or S phase of the cell cycle. Staining for **annexin** V, which binds **phosphatidylserine** on the plasma membrane, as an early indicator of **apoptosis** and measurement of the activity of caspase-3, a signaling molecule in apoptotic pathways, indicated that C81 is a strong inducer of **apoptosis**. Expression of C81 induced the condensation, fragmentation, and clumping of chromatin that are typical of **apoptosis**. Furthermore, the kinetics of the C81-induced G1 arrest were closely correlated with changes in the number of **annexin** V-positive cells and the activity of caspase-3. Replacement of Ile or Leu residues by Pro at positions 60, 67, 74, and 81 within the leucine zipper-like domain of C81 revealed that Ile60, Leu67, and Ile74 play important roles both in the C81-induced G1 arrest and in **apoptosis** . Thus, it appears that C81 induces **apoptosis** through pathways

that are identical to those utilized for G1 arrest of the cell cycle. It has been reported that Ile60, Leu67, and Ile74 also play an important role in the C81-induced suppression of growth. These results suggest that the suppression of growth induced by C81 result in **apoptosis** that is independent of G2 arrest of the cell cycle.

L9 ANSWER 65 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:271660 BIOSIS
DN PREV200000271660
TI Cell-surface exposure of **phosphatidylserine** correlates with the stage of fludarabine-induced **apoptosis** in chronic lymphocytic leukemia and expression of **apoptosis**-regulating genes.
AU Clodi, Katharina; Kliche, Kay Oliver; Zhao, Shourong; Weidner, Douglas; Schenk, Thomas; Consoli, Ugo; Jiang, Shuwei; Snell, Virginia; Andreeff, Michael (1)
CS (1) Department of Molecular Hematology and Therapy, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX, 77030 USA
SO Cytometry, (May 1, 2000) Vol. 40, No. 1, pp. 19-25. print..
ISSN: 0196-4763.
DT Article
LA English
SL English
AB Background: Programmed cell death (PCD) is characterized by a sequence of tightly regulated events that result in the activation of caspases and in internucleosomal DNA cleavage. Late apoptotic events such as DNA-strand breaks can be assayed by in situ end labeling (ISEL) and DNA measurement (sub G1) using flow cytometry. **Phosphatidylserine** (PS) redistribution from the inner plasma membrane leaflet to the outer leaflet, an early event in PCD, can be detected by **annexin** V (AxV) binding to PS. AxV-fluorescein isothiocyanate (FITC) fluorescence intensity is variable and characterizes different cell populations, denoted here as AxV-negative (AxVneg), AxV-low-positive (AxVlo), and AxV-high-positive (AxVhi). Methods: We investigate the correlation of three methods (ISEL, sub G1 DNA content, and AxV assay) for detecting **apoptosis** with focus on differences between populations with different levels of PS. We also examined the expression of PCD-regulating Bcl-2 family members in these cell populations by reverse transcription-polymerase chain reaction (RT-PCR). Chronic lymphocytic leukemia (CLL) cells exposed to fludarabine (FAMP) were used as an in vitro model. Cells with different PS/AxV levels were separated using fluorescence-activated cell sorting (FACS). Results: Only purified AxVhi cells had high positivity in the ISEL and sub G1 assays (94 +- 0.6%, 88.6 +- 6.6%, and 98.6 +- 0.6%, respectively), indicating that late apoptotic cells are detected equally by all three methods. In the AxVlo population, ISEL was positive in 21% +- 13% and DNA sub G1 in 20% +- 6.6% of cells, suggesting that AxV identifies early apoptotic cells better than the other assays. Anti-apoptotic Bcl-2 and Bcl-XL were upregulated by FAMP when cells entered **apoptosis** (AxVlo), as was pro-apoptotic Bcl-Xs, which was undetectable in nonapoptotic AxVneg cells. Pro-apoptotic Bax was only expressed in AxVneg and AxVlo cells. Late apoptotic AxVhi cells did not express Bcl-Xs or Bax. Results: (1) AxV staining is more sensitive than sub G1 or ISEL in detecting early apoptotic cells; (2) only late apoptotic cells are equally detected by all assays; (3) AxV is a valuable tool in the detection and isolation of apoptotic cells at different stages of PCD; and (4) pro-apoptotic Bcl-Xs and Bax are expressed at early, not late, stages of **apoptosis**.

L9 ANSWER 66 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:271659 BIOSIS
DN PREV200000271659
TI There is substantial nuclear and cellular disintegration before detectable **phosphatidylserine** exposure during the camptothecin-induced **apoptosis** of HL-60 cells.

AU King, Malcolm A. (1); Radicchi-Mastroianni, Monica A.; Wells, John V.
CS (1) Department of Clinical Immunology, Royal North Shore Hospital, Saint Leonards, NSW, 2065 Australia
SO Cytometry, (May 1, 2000) Vol. 40, No. 1, pp. 10-18. print..
ISSN: 0196-4763.
DT Article
LA English
SL English
AB Background: An early sign of **apoptosis** in many cells is the appearance of **phosphatidylserine** (PS) on the outside of the plasma membrane, whilst the cells still retain the ability to exclude DNA-binding molecules such as propidium iodide and 7-aminoactinomycin D (7-AAD). The protein **annexin** V binds preferentially to PS and has often been used to monitor the early phase of **apoptosis**. There have been some conflicting results concerning whether **annexin** V binds to camptothecin (CAM)-treated HL-60 cells, a commonly used model for **apoptosis**. We investigated the effects of culturing HL-60 cells for up to 8 h with a range of CAM concentrations. Methods: We used flow cytometry to measure cellular light scatter, **annexin** V-FITC binding, and 7-AAD uptake, and DNA content after fixation and permeabilization. We also used microscopy to examine the morphology of cells (both unsorted and sorted according to their light scatter) after cytocentrifugation. Results: We found that CAM caused the rapid appearance of low light scatter apoptotic bodies. Even among cells with "normal" light scatter, there was widespread DNA cleavage and nuclear fragmentation by 3 h. The percentage of apoptotic bodies peaked at about 4 h and it was only afterward that **annexin** V binding could be detected to both intact cells and to apoptotic bodies. When they first appeared, the intact **annexin** V+ cells had S-phase DNA content. Conclusions: During CAM-induced **apoptosis** of HL-60 cells, the external exposure of PS can either precede or follow DNA cleavage, which suggests that PS exposure is not always an indicator of early **apoptosis**.

L9 ANSWER 67 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:254760 BIOSIS
DN PREV200000254760
TI Changes in intercellular junctions during **apoptosis** precede nuclear condensation or **phosphatidylserine** exposure on the cell surface.
AU Corfe, B. M. (1); Dive, C.; Garrod, D. R.
CS (1) School of Biological Sciences, University of Manchester, Oxford Road, Manchester, M13 9PT UK
SO Cell Death and Differentiation, (Feb., 2000) Vol. 7, No. 2, pp. 234-235. print..
ISSN: 1350-9047.
DT Letter
LA English
SL English

L9 ANSWER 70 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:228072 BIOSIS
DN PREV200000228072
TI **Apoptosis**: The importance of nuclear medicine.
AU Blankenberg, F. G.; Tait, J.; Ohtsuki, K.; Strauss, H. W. (1)
CS (1) Department of Radiology/Division of Nuclear Medicine, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA, 94305-5105 USA
SO Nuclear Medicine Communications, (March, 2000) Vol. 21, No. 3, pp. 241-250.
ISSN: 0143-3636.
DT Article
LA English
SL English

AB **Apoptosis** is a genetically controlled, energy-dependent process which removes unwanted cells from the body. Because of its orderly progression, **apoptosis** is also known as programmed cell death or cell suicide. Once initiated, **apoptosis** is characterized by a series of biochemical and morphological changes involving the cytoplasm, nucleus and cell membrane. Cytoplasmic changes include cytoskeletal disruption, cytoplasmic shrinkage and condensation; prominent changes in the nucleus include peripheral chromatin clumping and inter-nucleosomal DNA cleavage (DNA ladder formation); and membrane changes include the expression of **phosphatidylserine** on the outer surface of the cell membrane and blebbing (resulting in the formation of cell membrane-bound vesicles or apoptotic bodies). These events allow the cell to digest and package itself into membrane-bound packets containing autodigested cytoplasm and DNA, which can then be easily absorbed by adjacent cells or phagocytes. An endogenous human protein, **annexin** V (molecular weight approximately 35,000), has an affinity of about 10-9 M for **phosphatidylserine** exposed on the surface of apoptotic cells. **Annexin** V can be labelled with radionuclides such as iodine or technetium, or positron emitting agents. Experimental studies in cells confirm that fluorescence and 99Tcm-labelled **annexin** have comparable affinity for apoptotic cells. In vivo studies with 99Tcm-labelled **annexin** confirm that radiolabelled **annexin** V can be used to image apoptotic cells/tissues in vivo. In this article, we review experimental data using **annexin** V imaging and discuss its possible future use to identify **apoptosis** in vivo.

L9 ANSWER 71 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:184647 BIOSIS
DN PREV200000184647
TI Comparison of DiOC6(3) uptake and **annexin** V labeling for quantification of **apoptosis** in leukemia cells and non-malignant T lymphocytes from children.
AU Ozgen, Unsal; Savasan, Sureyya (1); Buck, Steven; Ravindranath, Yaddanapudi
CS (1) Division of Hematology/Oncology, Children's Hospital of Michigan, Detroit, MI, 48201 USA
SO Cytometry, (Feb. 15, 2000) Vol. 42, No. 1, pp. 74-78. ISSN: 0196-4763.
DT Article
LA English
SL English
AB Early during **apoptosis**, there is a reduction in mitochondrial transmembrane potential (MTP) and externalization of **phosphatidylserine** (PS) in cell membrane prior to eventual cell death. Flow cytometric detection techniques targeting these changes, reduction of DiOC6(3) uptake upon the collapse of MTP and **annexin** V binding to PS have been successfully used to detect apoptotic cells. These methods have given comparable results when cell lines were used. We compared the two different techniques, DiOC6(3) uptake and **Annexin** V-propidium iodide co-labeling in the quantification of cytarabine, vincristine and daunorubicin induced **apoptosis** on three leukemia cell lines (HL-60, CEM, U937), and bone marrow blasts from 26 children with acute myeloid leukemia, 14 with T cell acute lymphoblastic leukemia. Anti-Fas-induced **apoptosis** in culture-grown peripheral blood T lymphocytes on 18 samples from 9 children with non-malignant conditions were also studied by these techniques. Our results showed that there is a correlation ($P < 0.05$) between the **apoptosis** rates measured by these two techniques for drug-induced **apoptosis** in myeloid and lymphoid blasts, and for anti-Fas mAb-induced **apoptosis** in T lymphocytes. This data suggests that reduction of the MTP and PS externalization may be common to many apoptotic pathways and techniques targeting either of these changes may be used in quantification of **apoptosis** in different clinical samples.

L9 ANSWER 72 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:176788 BIOSIS
DN PREV200000176788
TI Bcl-2, survivin and variant CD44 v7-v10 are downregulated and p53 is upregulated in breast cancer cells by progesterone: Inhibition of cell growth and induction of **apoptosis**.
AU Formby, B. (1); Wiley, T. S.
CS (1) Sansum Medical Research Institute, 2219 Bath Street, Santa Barbara, CA, 93105 USA
SO Molecular and Cellular Biochemistry., (Dec., 1999) Vol. 202, No. 1-2, pp. 53-61.
ISSN: 0300-8177.
DT Article
LA English
SL English
AB Progesterone inhibits the proliferation of normal breast epithelial cells in vivo, as well as breast cancer cells in vitro. But the biologic mechanism of this inhibition remains to be determined. We explored the possibility that an antiproliferative activity of progesterone in breast cancer cell lines is due to its ability to induce **apoptosis**. Since p53, bcl-2 and survivin genetically control the apoptotic process, we investigated whether or not these genes could be involved in the progesterone-induced **apoptosis**. We found a maximal 90% inhibition of cell proliferation with T47-D breast cancer cells after exposure to 10 muM progesterone for 72 h. Control progesterone receptor negative MDA-231 cancer cells were unresponsive to 10 muM progesterone. The earliest sign of **apoptosis** is translocation of **phosphatidylserine** from the inner to the outer leaflet of the plasma membrane and can be monitored by the calcium-dependent binding of **annexin** V in conjunction with flow cytometry. After 24 h of exposure to 10 muM progesterone, cytofluorometric analysis of T47-D breast cancer cells indicated 43% were **annexin** V-positive and had undergone **apoptosis** and no cells showed signs of cellular necrosis (propidium iodine negative). After 72 h of exposure to 10 muM progesterone, 48% of the cells had undergone **apoptosis** and 40% were **annexin** V positive/propidium iodide positive indicating signs of necrosis. Control untreated cancer cells did not undergo **apoptosis**. Evidence proving **apoptosis** was also demonstrated by fragmentation of nuclear DNA into multiples of oligonucleosomal fragments. After 24 h of exposure of T47-D cells to either 1 or 10 muM progesterone, we observed a marked down-regulation of protooncogene bcl-2 protein and mRNA levels. mRNA levels of survivin and the metastatic variant CD44 v7-v10 were also downregulated. Progesterone increased p53 mRNA levels. These results demonstrate that progesterone at relative high physiological concentrations, but comparable to those seen in plasma during the third trimester of human pregnancy, exhibited a strong antiproliferative effect on breast cancer cells and induced **apoptosis**.

L9 ANSWER 73 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:176160 BIOSIS
DN PREV200000176160
TI Mechanisms of cyclosporine A-induced **apoptosis** in rat hepatocyte primary cultures.
AU Grub, Sibylle; Persohn, Elke; Trommer, Wolfgang E.; Wolf, Armin (1)
CS (1) Novartis Pharma AG, WSH-2881.3.27, CH-4002, Basel Switzerland
SO Toxicology and Applied Pharmacology., (March 15, 2000) Vol. 163, No. 3, pp. 209-220.
ISSN: 0041-008X.
DT Article
LA English
SL English
AB In rat hepatocytes and isolated liver mitochondrial fractions,

Cyclosporine A (CsA) is often used as a specific inhibitor of mitochondrial Ca2+ release and as a specific blocker of mito-chondrial membrane potential and permeability transition (MPT), which are all processes involved in the inhibition of **apoptosis**. However, neither inhibition nor induction of **apoptosis** by CsA has yet been described in the rat hepatocyte primary culture during incubation for 4 and 20 h. It was the purpose of the present study to examine by means of morphological and biochemical criteria the effects of CsA on **apoptosis** and to characterize the underlying mechanisms. Rat hepatocytes were cultured for 4 or 20 h with CsA at concentrations of 0, 10, 25, and 50 muM. Chromatin condensation and fragmentation, DNA fragmentation (TUNEL), membrane **phosphatidylserine** distribution (**Annexin** V), caspase-1, -3, and -6 activity, mitochondrial membrane potential (Rhodamine 123), and cytochrome c release into the cytosol were investigated. Four hours after CsA treatment, chromatin condensation and fragmentation and the number of TUNEL- and **Annexin** V-positive cells increased dose-dependently without any observable enzyme leakage, which indicated the integrity of the outer cell membrane. After 20 h of CsA incubation **apoptosis** parameters were further increased and were accompanied by the increased activity of the cysteine protease, caspase-3 (CPP 32), and slightly increased caspase-6 (Mch 2), but not caspase-1 (ICE). The caspase-3 inhibitor, Ac-DEVD-CHO, inhibited caspase-3 activation and attenuated CsA-induced **apoptosis** and LDH leakage. The caspase-6 inhibitor, Ac-VEID-CHO, only marginally inhibited CsA-induced **apoptosis**. Decreased mitochondrial membrane potential and cytochrome c release went in parallel with ultrastructural mitochondrial changes and might be regarded as early events that trigger the **apoptosis** cascade. Transmission electron microscopy confirmed an increase in the number of necrotic cells after 20 h, but not after 4 h, compared with controls.

L9 ANSWER 76 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:123304 BIOSIS
DN PREV200000123304
TI Fluorescein fluorescence hyperpolarization as an early kinetic measure of the apoptotic process.
AU Zurgil, Naomi; Schiffer, Zeev; Shafran, Yana; Kaufman, Menachem; Deutsch, Mordechai (1)
CS (1) Jerome Schottenstein Cellscan Center for Early Detection of Cancer, Physics Department, Bar-Ilan University, Ramat-Gan, 52900 Israel
SO Biochemical and Biophysical Research Communications, (Feb. 5, 2000) Vol. 268, No. 1, pp. 155-163.
ISSN: 0006-291X.
DT Article
LA English
SL English
AB The ability to identify apoptotic cells within a complex population is crucial in the research and diagnosis of normal physiology and disease states. The Cellscan mark S (CS-S) cytometer was used in this study to detect intracellular fluorescence intensity and polarization (FI and FP) in several well-established models of **apoptosis**: Following spontaneous **apoptosis**, as well as glucocorticoid or anti Fas-induced **apoptosis**, CS-S individual cell-based analysis revealed the appearance of a cell cluster characterized by low FI and high FP. Temporal analysis of annexine V binding and FP measurements following DXM treatment showed that hyperpolarization preceded **phosphatidylserine** appearance on the outer plasma membrane. The early increase in FP was found to be dose dependent and inversely related to cell diameter. Cell dehydration and alteration of plasma membrane transport properties, both occurring during early stages of **apoptosis**, may be involved in the phenomena of intracellular fluorescein hyper-polarization in **apoptosis**.

L9 ANSWER 78 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AN 2000:83535 BIOSIS
DN PREV200000083535
TI Degradation of apoptotic cells and fragments in HL-60 suspension cultures after induction of **apoptosis** by camptothecin and ethanol.
AU Baisch, H. (1); Bollmann, H.; Bornkessel, S.
CS (1) Institute of Biophysics and Radiobiology, University of Hamburg, Martinistrasse 52, 20246, Hamburg Germany
SO Cell Proliferation, (oct., 1999) Vol. 32, No. 5, pp. 303-319.
ISSN: 0960-7722.
DT Article
LA English
SL English
AB Early indicators of **apoptosis** in mammalian cells are membrane potential breakdown (loss) in mitochondria (MPLM), chromatin condensation, DNA degradation, and **phosphatidylserine** exposure (PSE) on the outside plasma membrane. One aim of the present study was to determine the kinetics of these characteristics. These changes were measured by flow cytometry using the following methods: membrane potential of mitochondria was analysed using Mito Tracker Green and Red, PSE was analysed using **annexin**-V-FITC staining simultaneously with propidium iodide (PI) to detect membrane permeability; chromatin condensation was measured using the acid denaturation Acridine Orange (AO) method; DNA degradation was studied by the sub G1 method and the terminal transferase dUTP nick end-labelling (TUNEL) assay (labelling of strand breaks). HL-60 cells were induced to **apoptosis** by 3% ethanol and 1.5 muM camptothecin (CAM) and the kinetics of the apoptotic cells were measured. The same kinetics were found for chromatin condensation and DNA degradation indicating that these changes appeared at approximately the same time after induction. The MPLM and PSE kinetics showed a considerably later increase indicating that MPLM occurred downstream of DNA degradation and that plasma membrane changes occurred downstream of MPLM. The main aim of the study was to follow the fate of apoptotic cells after the appearance of the initial characteristics. The lifetime of apoptotic cells was studied by chase experiments. The inducing drug was removed after 4 h treatment and the disappearance of apoptoses recorded. An exponential decay was measured with a half life (T1/2) of 17.8 h. As a corollary from these experiments, camptothecin was found to induce **apoptosis** also in G1 and G2 phase cells, however, it took much longer to occur than in S phase cells. Using labelling of the plasma membrane with a fluorescent cell membrane linker, it was possible to show that the majority of apoptotic bodies as well as condensed apoptotic cells contain DNA and membrane. The degradation of these apoptotic bodies follows similar kinetics as those of the condensed apoptotic cells. The membrane remained considerably stable, there was no further loss in the next 7 days, after the first day when the apoptotic characteristics develop. It is concluded that the **apoptosis** programme is completed within a day and no further steps follow.

L9 ANSWER 81 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:50199 BIOSIS
DN PREV200000050199
TI Hoechst 33342 induces **apoptosis** in HL-60 cells and inhibits topoisomerase I in vivo.
AU Zhang, Xinbo; Chen, Jenn; Davis, Bruce; Kiechle, Frederick (1)
CS (1) Department of Clinical Pathology, 3601 W 13 Mile Rd, Royal Oak, MI USA
SO Archives of Pathology & Laboratory Medicine, (Oct., 1999) Vol. 123, No. 10, pp. 921-927.
ISSN: 0363-0153.
DT Article
LA English
SL English
AB Context: Bisbenzimides (Hoechst 33342 and Hoechst 33258) are cell-permeable, adenine-thymine-specific dyes that bind to the minor groove of DNA and stain DNA. Hoechst 33342 induces **apoptosis** in

BC3H-1 myocytes and hepatoma cells. Objective: To determine if Hoechst 33342 or Hoechst 33258 induces **apoptosis** in human promyelocytic leukemia cells (HL-60) and inhibits topoisomerase I activity. Design: A variety of methods were used to detect **apoptosis**: cell viability (trypan blue exclusion), nuclear fluorescence staining (Hoechst 33342 or Hoechst 33258 stained for 10 minutes), flow cytometric quantitation of **annexin** binding to **phosphatidylserine**, and DNA fragmentation (agarose gel electrophoresis). Topoisomerase I activity was determined by a plasmid unwinding assay. Setting: A large teaching hospital and research laboratories. Patients: None. Intervention: None. Main Outcome Measurements: **Apoptosis** is characterized by decreased cell viability, condensation of nuclear chromatin, increased **phosphatidylserine** translocation, and DNA fragmentation into oligonucleosomes composed of multiples of 180 to 200 base pairs. Inhibition of endogenous nuclear topoisomerase I is detected by the absence of plasmid unwinding from a tightly coiled to relaxed form. Results: Hoechst 33342, but not Hoechst 33258, induced **apoptosis** in the HL-60 cells in a time- and dose-dependent manner. Endogenous nuclear topoisomerase I activity in HL-60 cells was inhibited by treatment with Hoechst 33342 but not Hoechst 33258. Conclusion: Hoechst 33342-induced HL-60 cell **apoptosis** may be related to the dye's inhibition of topoisomerase I activity.

L9 ANSWER 83 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:27639 BIOSIS
DN PREV200000027639
TI Automatic image analysis for quantification of **apoptosis** in animal cell culture by **annexin**-V affinity assay.
AU Plasier, B.; Lloyd, D. R.; Paul, G. C.; Thomas, C. R.; Al-Rubeai, M. (1)
CS (1) SERC Centre for Bioprocess Engineering, School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham, B15 2TT UK
SO Journal of Immunological Methods, (Oct. 29, 1999) Vol. 229, No. 1-2, pp. 81-95.
ISSN: 0022-1759.
DT Article
LA English
SL English
AB **Apoptosis** is a form of cell death in which the dying cell plays an active part in its demise. At the morphological level, it is characterised by cell shrinkage rather than the swelling seen in necrotic cell death. In cell culture, **apoptosis** limits the yield of economically and medically important products, and can result in synthesis of imperfect molecules. Therefore, this process must be identified, monitored and fully understood, so that a means to regulate it can be developed. We have developed a new automatic image analysis assay for detecting **apoptosis** in animal cell culture on the basis of the **annexin**-V affinity assay. The results of this assay were compared with data generated by flow cytometry and manual scoring. All three methods were found to correspond well but image analysis like flow cytometry offers operator-independent results, and can be used as a tool for rapid monitoring of viable cell number, **apoptosis** and necrosis in animal cell culture. Furthermore, reduction in cell size was measured and was found to precede the appearance of **phosphatidylserine** on the cell surface.

L9 ANSWER 85 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:14990 BIOSIS
DN PREV200000014990
TI A novel 96-well scintillation proximity assay for the measurement of **apoptosis**.
AU McMurtrey, Amy E. (1); Graves, Robert J.; Hooley, Jeff; Brophy, Gerard; Lewis Phillips, Gail D.
CS (1) Genentech, Inc., 1 DNA Way, South San Francisco, CA, 94080 USA
SO Cytotechnology, (1999) Vol. 31, No. 3, pp. 271-282.

ISSN: 0920-9069.
DT Article
LA English
SL English
AB The translocation of phospholipids across the plasma membrane has been widely documented as one of the earliest measurable biochemical events of **apoptosis**. Using fluorescently labelled **annexin** V, which preferentially binds **phosphatidylserine** (PS) in the presence of Ca2+, the externalization of PS can be measured and **apoptosis** quantified using flow cytometry. Conventional detection methods utilizing **annexin** V, while faster than in situ DNA end-labelling or DNA laddering, require extensive sample preparation which may compromise samples and makes rapid, high volume screening prohibitive. This paper describes a novel assay for the measurement of **apoptosis** based upon binding of radiolabelled **annexin** V to apoptotic cells attached to the growth surface of a 96-well scintillating microplate (Cytostar-T(R)). We compared measurements of **apoptosis** made by flow cytometry to those obtained with the scintillating microplate in three model systems, treatment of: mouse connective tissue (L-M) cells with lymphotoxin (LT), human lung carcinoma (H460) cells with Apo-2 ligand and human umbilical vein endothelial (HUVE) cells with staurosporine. In this assay, we compare both direct and indirect labelling methods by utilizing either iodinated **annexin** V or biotinylated **annexin** V/(35S) streptavidin to radiolabel apoptotic cells. The signal detected is a direct consequence of the binding of **annexin** V to externalized PS on apoptotic cells and the proximity of the label to the base of the plate. Using this method, separation of bound and unbound radiolabel signal occurs directly within the well resulting in a sensitive assay that requires minimal manipulation and can accomodate a large number of samples.

L9 ANSWER 86 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:11852 BIOSIS
DN PREV200000011852
TI TBT-induced **apoptosis** in tunicate haemocytes.
AU Cima, Francesca (1); Ballarin, Loriano
CS (1) Dipartimento di Biologia, Universita di Padova, Via U. Bassi 58/B, 35131, Padova Italy
SO Applied Organometallic Chemistry, (Oct., 1999) Vol. 13, No. 10, pp. 697-703.
ISSN: 0268-2605.
DT Article
LA English
SL English
AB Early events in **apoptosis** include chromatin condensation followed by DNA fragmentation as well as translocation of **phosphatidylserine** (PS) in the outer plasma membrane. Organotin compounds increase intracellular Ca2+ levels and cause **apoptosis** in mammalian cells. In investigating whether TBT may also induce **apoptosis** in haemocytes of the ascidian Botryllus schlosseri, we exposed haemocytes to this xenobiotic at the sublethal dose of 10 muM, causing cell shrinkage and inhibition of phagocytosis and respiratory burst. **Apoptosis** was revealed as (i) chromatin condensation, with Acridine Orange nuclear staining; (ii) DNA fragmentation, with the TUNEL reaction; (iii) PS translocation, with the **annexin**-V assay; and (iv) loss of membrane permeability with the Trypan Blue diffusion assay. After 1 h of exposure, nuclear changes, i.e. significant collapse and cleavage of chromatin, were observed and cytoplasm blebbing occurred, together with surface alterations triggered by PS exposure. Haemocyte mortality increased significantly only after 2 h. All these apoptotic events may be closely related to a TBT-induced cytosolic calcium increase resulting in activation of endonucleases.

L9 ANSWER 91 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AN 1999:490390 BIOSIS
DN PREV199900490390
TI Early detection of staurosporine-induced **apoptosis** by comet and **annexin** V assays.
AU Godard, Thierry; Deslandes, Edwige; Lebailly, Pierre; Vigreux, Carole; Sichel, Francois; Poul, Jean-Michel; Gauduchon, Pascal (1)
CS (1) Laboratoire de Cancerologie Experimentale, Centre Francois Baclesse, Route de Lion-sur-Mer, F-14076, Caen Cedex, 05 France
SO Histochemistry and Cell Biology, (Aug., 1999) Vol. 112, No. 2, pp. 155-161.
ISSN: 0948-6143.
DT Article
LA English
SL English
AB Comet, TUNEL, and **annexin** V assays were used to identify DNA fragmentation and plasma membrane alterations occurring during staurosporine-induced **apoptosis** in Chinese hamster ovary cells. TUNEL assay detected apoptotic cells after 6 h treatment. The occurrence of **annexin** V immunofluorescence staining after 1 h treatment confirms that exposure of **phosphatidylserine** (PS) residues is an early biochemical feature of **apoptosis**. According to intensity, three **annexin** staining patterns were distinguished, related to different steps in the apoptotic process. The detection of highly damaged cells by the comet assay after 3 h treatment occurred earlier than the detection of DNA modifications by the TUNEL assay, but later than the exposure of PS residues. However, late apoptotic cells, otherwise characterized by plasma membrane disruption and high **annexin** V staining, were not detected by the comet assay. In this case, comet assay modified by omitting electrophoresis (halo assay) was more sensitivefor an accurate quantification of the apoptotic fraction.

L9 ANSWER 95 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:444581 BIOSIS
DN PREV199900444581
TI **Annexin** V staining due to loss of membrane asymmetry can be reversible and precede commitment to apoptotic death.
AU Hammill, Adrienne K.; Uhr, Jonathan W.; Scheuermann, Richard H. (1)
CS (1) Department of Pathology and Laboratory of Molecular Pathology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX, 75235 USA
SO Experimental Cell Research, (Aug. 25, 1999) Vol. 251, No. 1, pp. 16-21.
ISSN: 0014-4827.
DT Article
LA English
SL English
AB Signal-induced **apoptosis** is a normal phenomenon in which cells respond to changes in their environment through a cascade of intracellular biochemical changes culminating in cell death. However, it is not clear at what point in this process the cell becomes committed to die. An early biochemical change characteristic of cells undergoing **apoptosis** is the loss of plasma membrane asymmetry, such that high levels of **phosphatidylserine** become exposed on the outside cell surface. These cells can be recognized by staining with **Annexin** V, which binds to **phosphatidylserine** with high affinity. To investigate the mechanisms controlling signal-induced **apoptosis** we have examined the response of a B cell lymphoma to crosslinking of the membrane immunoglobulin (mIg) receptor. We have found that many of the cells that stain positive for **Annexin** V are viable and can resume growth and reestablish phospholipid asymmetry once the signal is removed. These results indicate that **Annexin** V staining, and thus loss of membrane asymmetry, precedes commitment to apoptotic death in this system.

L9 ANSWER 96 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:430526 BIOSIS

DN PREV199900430526
TI Increased cell surface exposure of **phosphatidylserine** on propidium iodide negative thymocytes undergoing death by necrosis.
AU Waring, Paul (1); Lambert, Damaris; Sjaarda, Allan; Hurne, Alanna; Beaver, Joanne
CS (1) Division of Immunology and Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra Australia
SO Cell Death and Differentiation, (July, 1999) Vol. 6, No. 7, pp. 624-637. ISSN: 1350-9047.
DT Article
LA English
SL English
AB **Phosphatidylserine** (PS) exposure on propidium iodide negative cells using FITC labelled **annexin**-V has been used to quantify **apoptosis** in vitro and in vivo. Detection of PS within cells undergoing necrosis is also possible if labelled **annexin**-V specific for PS enters the cell following early membrane damage. Necrotic or late apoptotic cells can be excluded from flow cytometric analysis using propidium iodide which enters and stains cells with compromised membrane integrity. Here we show that thymocytes undergoing death exclusively by necrosis show early exposure of PS prior to loss of membrane integrity. This early exposure of PS occurs in cells treated with agents which both raise intracellular calcium levels and are also capable of interacting with protein thiol groups. We also demonstrate that PS exposure in thymocytes induced to undergo **apoptosis** by three different agents does not correlate with calcium rises but correlates with and precedes DNA fragmentation.

L9 ANSWER 102 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:325473 BIOSIS
DN PREV199900325473
TI Release of mitochondrial cytochrome C in both **apoptosis** and necrosis induced by beta-lapachone in human carcinoma cells.
AU Li, You-Zhi; Li, Chiang J. (1); Pinto, Antonio Ventura; Pardee, Arthur B.
CS (1) Dana-Farber Cancer Institute, D612, 44 Binney St., Boston, MA, 02115 USA
SO Molecular Medicine (New York), (April, 1999) Vol. 5, No. 4, pp. 232-239. ISSN: 1076-1551.
DT Article
LA English
SL English
AB Background: There are two fundamental forms of cell death: **apoptosis** and necrosis. Molecular studies of cell death thus far favor a model in which **apoptosis** and necrosis share very few molecular regulators. It appears that apoptotic processes triggered by a variety of stimuli converge on the activation of a member of the caspase family, such as caspase 3, which leads to the execution of **apoptosis**. It has been suggested that blocking of caspase activation in an apoptotic process may divert cell death to a necrotic demise, suggesting that **apoptosis** and necrosis may share some upstream events. Activation of caspase is preceded by the release of mitochondrial cytochrome C. Materials and Methods: We first studied cell death induced by beta-lapachone by MTT and colony-formation assay. To determine whether the cell death induced by beta-lapachone occurs through necrosis or **apoptosis**, we used the PI staining procedure to determine the sub-G1 fraction and the **Annexin**-V staining for externalizationof phophatidylserine. We next compared the release of mitochondrial cytochrome C in **apoptosis** and necrosis. Mitochondrial cytochrome C was determined by Western blot analysis. To investigate changes in mitochondria that resulted in cytochrome C release, the mitochondrial membrane potential (delta psi) was analyzed by the accumulation of rhodamine 123, a membrane-permeant cationic fluorescent dye. The activation of caspase in **apoptosis** and necrosis were measured by using a profluorescent substrate for caspase-like proteases,

PhiPhiLuxG6D2. Results: beta-lapachone induced cell death in a spectrum of human carcinoma cells, including nonproliferating cells. It induced **apoptosis** in human ovary, colon, and lung cancer cells, and necrotic cell death in four human breast cancer cell lines. Mitochondrial cytochrome C release was found in both **apoptosis** and necrosis. This cytochrome C release occurred shortly after beta-lapachone treatment when cells were fully viable by trypan blue exclusion and MTT assay, suggesting that cytochrome C release is an early event in beta-lapachone induced **apoptosis** as well as necrosis. The mitochondrial cytochrome C release induced by beta-lapachone is associated with a decrease in mitochondrial transmembrane potential (delta psi). There was activation of caspase 3 in apoptotic cell death, but not in necrotic cell death. This lack of activation of CPP 32 in human breast cancer cells is consistent with the necrotic cell death induced by beta-lapachone as determined by absence of sub-G1 fraction, externalization of **phosphatidylserine**. Conclusions: beta-lapachone induces either apoptotic or necrotic cell death in a variety of human carcinoma cells including ovary, colon, lung, prostate, and breast, suggesting a wide spectrum of anti-cancer activity in vitro. Both apoptotic and necrotic cell death induced by beta-lapachone are preceded by a rapid release of cytochrome C, followed by the activation of caspase 3 in apoptotic cell death but not in necrotic cell death. Our results suggest that beta-lapachone is a potential anti-cancer drug acting on the mitochondrial cytochrome C-caspase pathway, and that cytochrome C is involved in the early phase of necrosis.

L9 ANSWER 104 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:281379 BIOSIS
DN PREV199900281379
TI A novel method for measuring CTL and NK cell-mediated cytotoxicity using **annexin** V and two-color flow cytometry.
AU Goldberg, Jodi E.; Sherwood, Steven W.; Clayberger, Carol (1)
CS (1) Department of Cardiothoracic Surgery, Stanford University School of Medicine, Stanford, CA, 94305-5407 USA
SO Journal of Immunological Methods, (April 22, 1999) Vol. 224, No. 1-2, pp. 1-9.
ISSN: 0022-1759.
DT Article
LA English
SL English
AB An assay based on two-color flow cytometry has been developed to measure CTL and NK cell-mediated cytotoxicity. After effector/target cells are incubated together, CTL or NK populations are stained with an effector cell specific PE-conjugated mAb. Subsequently, **annexin** V-FITC binds to cells expressing **phosphatidylserine** (an early marker of **apoptosis**) on the cell surface. Target cells are gated upon as PE-negative and quantified with respect to their **annexin** V positivity. The shift from **annexin** Vneg to **annexin** Vhi is a discrete event such that all target cells fall within discernible populations with respect to **annexin** V. There is a strong correlation between cytotoxicity measured with our assay and a standard 51Cr release assay (r2 = 0.989). The PE/**annexin** V assay shows increased sensitivity at early timepoints after target/effector cell mixing. In addition, this method allows for analysis of target cells at the single cell level. Therefore, we have described a promising new technique to measure in vitro cell-mediated cytotoxicity. It avoids the potential difficulties of working with radioactive isotopes, and offers increased sensitivity and versatility.

L9 ANSWER 108 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:208063 BIOSIS
DN PREV199900208063
TI Flow cytometric scoring of **apoptosis** compared to electron microscopy in gamma irradiated lymphocytes.

AU Louagie, H. (1); Cornelissen, M.; Philippe, J.; Vral, A.; Thierens, H.; De Ridder, L.
CS (1) Department of Anatomy, Embryology and Histology Section Histology, University of Gent, Pasteurlaan 2, B 9000, Gent Belgium
SO Cell Biology International, (1998) Vol. 22, No. 4, pp. 277-283. ISSN: 1065-6995.
DT Article
LA English
SL English
AB One of the early events occurring at the cell membrane during **apoptosis** is the translocation of **phosphatidylserine** from the inner side of the plasma membrane to the outer layer. These **phosphatidylserine** groups can be bound by fluorescein isothiocyanate (FITC)-labelled **annexin** V. The aim of this study was to evaluate the power of the **annexin** V flow cytometric assay in detecting **apoptosis** in gamma irradiated peripheral blood lymphocytes and in differentiating between **apoptosis** and primary necrosis in these cells. Therefore, 5 Gy and 20 Gy gamma irradiated peripheral blood mononuclear cells (PBMCs) were examined after a 24-h culture period. The terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) technique was performed as well. A comparison with an electron microscopic (EM) evaluation was made. EM is based on established morphological criteria allowing the classification of cells into four groups: viable, early apoptotic, secondary necrotic and primary necrotic cells. EM performed on **annexin** V positive sorted cells proved that a 5 Gy gamma irradiation of PBMCs mainly causes **apoptosis**, whereas a 20 Gy gamma irradiation mainly induces primary necrosis. Neither the **annexin** V flow cytometric assay nor the TUNEL assay were able to distinguish between primary and secondary necrotic cells. These results illustrate that if quantification of **apoptosis** is required, one should be careful in interpreting flow cytometric results obtained by **annexin** V or TUNEL staining in peripheral blood lymphocytes. Although in general primary necrotic cells show an increased forward scatter due to cellullar swelling, both early apoptotic and necrotic (primary or secondary) lymphocytes show a decreased forward scatter signal. Moreover, both primary and secondary necrotic lymphocytes are **annexin** V and propidium iodide (PI) positive and therefore indistinguishable. We conclude that if a new experiment focusing on **apoptosis** is set up, an initial EM evaluation is mandatory. If EM shows that the **apoptosis** inducing agent used in the design of the experiments is not causing primary necrosis, than the **annexin** V flow cytometric assay can provide rapid and quantitative information about **apoptosis**.

L9 ANSWER 109 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:180175 BIOSIS
DN PREV199900180175
TI Histochemical demonstration of apoptotic cells in the chicken embryo using **annexin** V.
AU Schrevens, A.; Van Nassauw, L.; Harrisson, F.
CS Lab. Hum. Anatomy Embryology, Univ. Antwerp, 171 Groenenborgerlaan, B-2020 Antwerp Belgium
SO Histochemical Journal, (Dec., 1998) Vol. 30, No. 12, pp. 917-922. ISSN: 0018-2214.
DT Article
LA English
AB This study describes the use of biotinylated **annexin** V for the histochemical detection of apoptotic cells in cultured chicken embryos during gastrulation. This method is based on the Ca2+-dependent binding of **annexin** V to **phosphatidylserine**, a negatively charged phospholipid, located at the inner leaflet of the cell membrane in living cells. However, in the early stages of **apoptosis**, **phosphatidylserine** is translocated to the outer layer of the cell membrane and can then be recognized by **annexin** V. Applying this

method in cultured chicken embryos during gastrulation, we obtained labelling of apoptotic cells in the three germ layers. In the epiblast and mesoblast, labelling was predominantly present in the region lateral to the primitive streak. At the level of the germinal crescent, labelled cells were also found in the epiblast. Labelled cells in the deep layer, which is a heterogeneous tissue layer composed of endophyll, sickle endoblast and definitive endoblast, were rather scarce. The distribution of cells, as observed in this study after labelling with **annexin** V in light microscopy and confocal laser scanning microscopy, is consistent with distributions reported by other authors using other approaches and with our previous observations made with the TUNEL technique and by electron microscopy after fixation in a tannic acid-based fixative. The main advantages of this method over other more sophisticated methods is its easiness and rapidity of execution and the fact that both early and late stages of **apoptosis** are detected.

L9 ANSWER 110 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:170404 BIOSIS
DN PREV199900170404
TI Arsenic trioxide and melarsoprol induce **apoptosis** in plasma cell lines and in plasma cells from myeloma patients.
AU Rousselot, Philippe; Labaume, Sylvaine; Marolleau, Jean-Pierre; Larghero, Jerome; Noguera, Maria-Helena; Brouet, Jean-Claude; Fermand, Jean-Paul (1)
CS (1) Service d'Immuno-Hematologie, Hopital Saint-Louis, 1 Avenue Claude Vellefaux, 75475 Paris Cedex 10 France
SO Cancer Research, (March 1, 1999) Vol. 59, No. 5, pp. 1041-1048. ISSN: 0008-5472.
DT Article
LA English
AB Recent data have renewed the interest for arsenic-containing compounds as anticancer agents. In particular, arsenic trioxide (As2O3) has been demonstrated to be an effective drug in the treatment of acute promyelocytic leukemia by inducing programmed cell death in leukemic cells both in vitro and in vivo. This prompted us to study the in vitro effects of As2O3 and of another arsenical derivative, the organic compound melarsoprol, on human myeloma cells and on the plasma cell differentiation of normal B cells. At pharmacological concentrations (10-8 to 10-6 mol/L), As2O3 and melarsoprol caused a dose- and time-dependent inhibition of survival and growth in myeloma cell lines that was, in some, similar to that of acute promyelocytic leukemia cells. Both arsenical compounds induced plasma cell **apoptosis**, as assessed by 4',6-diamidino-2-phenylindole staining, detection of **phosphatidylserine** at the cell surface using **annexin** V, and by the terminal deoxynucleotidyl transferase-mediated nick end labeling assay. As2O3 and melarsoprol also inhibited viability and growth and induced **apoptosis** in plasma-cell enriched preparations from the bone marrow or blood of myeloma patients. In nonseparated bone marrow samples, both arsenical compounds triggered death in myeloma cells while sparing most myeloid cells, as demonstrated by double staining with **annexin** V and CD38 or CD15 antibodies. In primary myeloma cells as in cell lines, interleukin 6 did not prevent arsenic-induced cell death or growth inhibition, and no synergistic effect was observed with IFN-alpha. In contrast to As2O3, melarsoprol only slightly reduced the plasma cell differentiation of normal B cells induced by pokeweed mitogen. Both pokeweed mitogen-induced normal plasma cells and malignant plasma cells showed a normal nuclear distribution of PML protein, which was disrupted by As2O3 but not by melarsoprol, suggesting that the two arsenical derivatives acted by different mechanisms. These results point to the use of arsenical derivatives as investigational drugs in the treatment of multiple myeloma.

L9 ANSWER 115 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:104630 BIOSIS
DN PREV199900104630

TI Phospholipid rearrangement of apoptotic membrane does not depend on nuclear activity.
AU Reno, Filippo; Burattini, Sabrina; Rossi, Stefano; Luchetti, Francesca; Columbaro, Marta; Santi, Spartaco; Papa, Stefano; Falcieri, Elisabetta (1)
CS (1) Ist. Anat. and Fisiol., Univ. Urbino, I-61029 Urbino Italy
SO Histochemistry and Cell Biology, (Nov., 1998) Vol. 110, No. 5, pp. 467-476.
ISSN: 0948-6143.
DT Article
LA English
AB The behaviour of plasma membrane was studied in UV-treated cells to investigate its involvement in **apoptosis**. It was studied in HL60 cells, in which DNA oligonucleosomic cleavage occurs, and in Molt-4 cells, which are characterised by a different fragmentation pattern. During the early stages of **apoptosis**, a membrane lipid rearrangement occurs, which involves **phosphatidylserine** translocation from the inner to the outer leaflet. This molecular alteration was investigated by **annexin** VFITC binding, analysed by flow cytometry and confocal microscopy. It was correlated with transmission electron microscopy, subdiploid peak appearance and DNA fragmentation. Our data indicate that the plasma membrane represents an early apoptotic target, even if its alterations are not detectable by ultrastructural analysis, which indicates its good preservation until late apoptotic stages. In addition, the study of apoptotic cells with absent or inactivated endonuclease demonstrates the independence of this membrane mechanism from nuclear activity.

L9 ANSWER 116 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:98803 BIOSIS
DN PREV199900098803
TI Imaging of **apoptosis** (programmed cell death) with 99mTc **annexin** V.
AU Blankenberg, Francis G. (1); Katsikis, Peter D.; Tait, Jonathan F.; Davis, R. Eric; Naumovski, Louis; Ohtsuki, Katsuichi; Kopiwoda, Susan; Abrams, Michael J.; Strauss, H. W.
CS (1) Dep. Radiology, Stanford University School Medicine, 300 Pasteur Dr., Stanford, CA 94305 USA
SO Journal of Nuclear Medicine, (Jan., 1999) Vol. 40, No. 1, pp. 184-191.
ISSN: 0161-5505.
DT Article
LA English
AB **Apoptosis** (programmed cell death) is a critical element in normal physiology and in many disease processes. **Phosphatidylserine** (PS), one component of cell membrane phospholipids, is normally confined to the inner leaflet of the plasma membrane. Early in the course of **apoptosis**, this phospholipid is rapidly exposed on the cell's outer surface. **Annexin** V, an endogenous human protein, has a high affinity for membrane-bound PS. This protein has been labeled with fluorescein and has been used to detect **apoptosis** in vitro. We describe the use of radiolabeled **annexin** V to detect **apoptosis** in vivo. The results are compared to histologic and flow cytometric methods to identify cells and tissues undergoing **apoptosis**. Methods: **Annexin** V was coupled to hydrazinonicotinamide (HYNIC) and radiolabeled with 99mTc. Bioreactivity of 99mTc-HYNIC **annexin** V was compared with fluorescein isothiocyanate (FITC)-labeled **annexin** V in cultures of Jurkat T-cell lymphoblasts and in ex vivo thymic cell suspensions undergoing **apoptosis** in response to different stimuli. In addition, the uptake of FITC **annexin** V and 99mTc-HYNIC **annexin** V was studied in heat-treated necrotic Jurkat T-cell cultures. In vivo localization of **annexin** V was studied in Balb/c mice injected with 99mTc-HYNIC **annexin** V before and after induction of Fas-mediated hepatocyte **apoptosis** with intravenously administered antiFas antibody. Results: Membrane-bound

radiolabeled **annexin** V activity linearly correlated to total fluorescence as observed by FITC **annexin** V flow cytometry in Jurkat T-cell cultures induced to undergo **apoptosis** in response to growth factor deprivation (N = 10, r2 = 0.987), antiFas antibody (N = 8, r2 = 0.836) and doxorubicin (N = 10, r2 = 0.804); and in ex vivo experiments on thymic cell suspensions with dexamethasone-induced **apoptosis** from Balb/c mice (N=6, r2 = 0.989). Necrotic Jurkat T-cell cultures also demonstrated marked increases in radiopharmaceutical (4000-5000-fold) above control values. AntiFas antibody-treated Balb/c mice (N = 6) demonstrated a three-fold rise in hepatic uptake of **annexin** V (P < 0.0005) above control (N = 10), identified both by imaging and scintillation well counting. The increase in hepatic uptake in antiFas antibody-treated mice correlated to histologic evidence of fulminant hepatic **apoptosis**. Conclusion: These data suggest that 99mTc-HYNIC **annexin** V can be used to image apoptotic and necrotic cell death in vivo.

L9 ANSWER 119 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:63682 BIOSIS
DN PREV199900063682
TI Use of intracellular pH and **annexin**-V flow cytometric assays to monitor **apoptosis** and its suppression by bcl-2 over-expression in hybridoma cell culture.
AU Ishaque, Adiba; Al-Rubeai, Mohamed (1)
CS (1) Animal Cell Technology Group, Sch. Chemical Engineering, Univ. Birmingham, Birmingham B15 2TT UK
SO Journal of Immunological Methods, (Dec., 1998) Vol. 221, No. 1-2, pp. 43-57.
ISSN: 0022-1759.
DT Article
LA English
AB Accurate identification and quantitation of **apoptosis** is essential for developing efficient strategies for optimisation of culture viability and productivity in cell lines of industrial significance. We have examined the possibility of using carboxy-seminaphthorhodafluor-1-acetoxymethylester (carboxy SNARF-1-AM), a pH sensitive fluoroprobe and FITC-labelled **annexin** V (AV), a probe specific to **phosphatidylserine** exposed on the surface of apoptotic cells, to monitor **apoptosis** and to determine the relationship between intracellular pH (pHi), **apoptosis** and cell cycle in hybridoma cells. Temporal changes in the distribution of proliferative capacity (S phase), metabolic activity (pHi), and cell death population dynamics were effectively and reliably determined using flow cytometry. Intracellular acidification was shown to precede the occurrence of **apoptosis** during batch culture and after treatment with campothecin, staurosporine and under adverse bioreactor conditions such as glutamine deprivation and oxygen deficiency. These results showed that the decrease in pHi can be used as an indicator of cellular deterioration and cell death. AV in combination with propidium iodide permitted the identification of viable, transient apoptotic and necrotic cells in heterogeneous cultures of control (PEF) cells. Hybridoma cells over-expressing bcl-2 were protected from intracellular acidification and **phosphatidylserine** exposure, which was associated with the suppression of **apoptosis** in these cells. A decrease in pHi was apparent even before the accumulation of the normally acidic G1 phase and the development of-a-sub-G1 region, characteristic of apoptotic cell behaviour. The pHi assay can therefore be used as a tool to predict future cell culture performance.

L9 ANSWER 120 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:58348 BIOSIS
DN PREV199900058348
TI **Annexin** V binding assay as a tool to measure **apoptosis** in differentiated neuronal cells.

AU Schutte, B. (1); Nuydens, R.; Geerts, H.; Ramaekers, F.
CS (1) Dep. Mol. Cell Biol. Genet., Univ. Maastricht, P.O. Box 616, 6200 MD Maastrichts Netherlands
SO Journal of Neuroscience Methods, (Dec. 31, 1998) Vol. 86, No. 1, pp. 63-69.
ISSN: 0165-0270.
DT Article
LA English
AB We describe a rapid and reliable method to quantitate the extent of **apoptosis** in neuronal cell cultures. Based on their **annexin** V-affinity, resulting from **phosphatidylserine** (PS) exposure at the outer leaflet of the plasma membrane, apoptotic cells can be distinguished from **annexin** V-negative living cells, by using microscopic and flow cytometric procedures. When combined with propidium iodide (PI) the double labeling procedure allows a further distinction of necrotic (**annexin** V + /PI+), apoptotic (**annexin** V + /PI -) cells. Furthermore, when the cells are incubated with **annexin** V prior to harvesting, the former cell populations can be separated from cells damaged during isolation (**annexin** V - /PI +). In the present paper, we show that the **annexin** V-binding assay is also applicable to differentiated neuronal cells with fragile neurite outgrowths.

L9 ANSWER 122 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:36174 BIOSIS
DN PREV199900036174
TI **Apoptosis** and necrosis: Mechanisms of cell death induced by cyclosporine A in a renal proximal tubular cell line.
AU Healy, Edel; Dempsey, Mark; Lally, Christine; Ryan, Michael P. (1)
CS (1) Dep. Pharmacol., University Coll. Dublin, Fosters Ave., Blackrock, Co., Dublin Ireland
SO Kidney International, (Dec., 1998) Vol. 54, No. 6, pp. 1955-1966.
ISSN: 0085-2538.
DT Article
LA English
AB Background. The mechanisms of cyclosporine (CsA)-induced nephrotoxicity are not fully understood. While hemodynamic changes may be involved in vivo, there is also some evidence for tubular involvement. We previously showed direct toxicity of CsA in the LLC-PK1 renal tubular cell line. In the current study we examined mechanisms (**apoptosis** or necrosis) of cell death induced by CsA in the LLC-PK1 renal proximal tubular cell line. The possible role of the Fas (APO-1/CD95) antigen-Fas ligand system in the mediation of CsA-induced cell death was also investigated. Methods. Cells were treated with CsA (0.42 nM to 83 muM) for 24 hours and alterations in DNA and protein synthesis and membrane integrity were examined. Flow cytometry was used to investigate: (i) alterations in the DNA content and cell cycle; (ii) the forward (FSC) and side (SSC) light scattering properties (indicators of cell size and granularity, respectively); (iii) the externalization of **phosphatidylserine** (PS) as a marker of early **apoptosis** using FITC-**annexin** V binding; and (iv) expression of the apoptotic Fas protein. DNA fragmentation in apoptotic cells was also determined by the TUNEL assay. Results. CsA (all doses) caused a block in the G0/G1, phase of the cell cycle as indicated by a decrease in DNA synthesis and supported by an increase in the % of cells in the G0/G1 phase with concurrent decreases of those in the S and G2/M phases. The effect on protein synthesis appeared to be much less. Lower doses of CsA (4.2 nM) caused the appearance of a "sub-G0/G1" peak, indicative of reduced DNA content, on the DNA histogram that was paralleled by a reduction in cell size and an increased cell granularity and an increase in FITC-**annexin** V binding. DNA fragmentation was evident in these cells as assessed using the TUNEL assay. Higher doses of CsA increased cell size and decreased cell granularity and reduced membrane integrity. Expression of Fas, the cell surface molecule that stimulates **apoptosis**, was increased

following low dose CsA exposure. Conclusions. These results indicate that CsA is directly toxic to LLC-PK1 cells with reduced DNA synthesis and cell cycle blockade. The mode of cell death, namely **apoptosis** or necrosis, is dose dependent. Fas may be an important mediator of CsA induced **apoptosis** in renal proximal tubular cells.

L9 ANSWER 126 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:10605 BIOSIS
DN PREV199900010605
TI Comparison of seven quantitative assays to assess lymphocyte cell death during HIV infection: Measurement of induced **apoptosis** in anti-Fas-treated jurkat cells and spontaneous **apoptosis** in peripheral blood mononuclear cells from children infected with HIV.
AU McCloskey, Thomas W.; Chavan, Surendra; Lakshmi-Tamma, Seetha M.; Pahwa, Savita (1)
CS (1) North Shore Univ. Hosp., 350 Community Dr., Manhasset, NY 11030 USA
SO AIDS Research and Human Retroviruses, (Nov. 1, 1998) Vol. 14, No. 16, pp. 1413-1422.
ISSN: 0889-2229.
DT Article
LA English
AB The study of **apoptosis** in relation to various human disease states, particularly HIV infection, has seen a tremendous increase in activity. In this article, values obtained by seven different assays, designed to quantify **apoptosis** and applicable to the study of HIV infection, are compared in two cell systems: (1) stimulus-induced **apoptosis** in Jurkat cells treated with anti-Fas antibody and (2) spontaneous **apoptosis** in PBMCs isolated from HIV-infected children. The methods used included measurement of cells with subdiploid DNA content, labeling of DNA strand breaks by the TUNEL reaction, **annexin** V surface labeling for the detection of exposed **phosphatidylserine**, cytoplasmic antigen labeling with the **apoptosis**-specific antibody Apo 2.7, detection of changes in flow cytometric light-scattering properties, trypan blue dye exclusion by light microscopy, and detection of changes in cellular chromatin by fluorescence microscopy. These methods produced well-correlated values in the Jurkat system, whereas the same set of methods produced more discrepant values in of all the methods tested, showed excellent overall correlation in both cell systems, was highly specific, and matched microscopic observation of the cells. Although many of the methods were suited to the study of a homogeneous cell line, caution must be exercised when examining cell death in a heterogeneous cell mixture from an HIV-infected individual.

L9 ANSWER 132 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:473586 BIOSIS
DN PREV199800473586
TI Random versus selective membrane phospholipid oxidation in **apoptosis**: Role of **phosphatidylserine**.
AU Fabisiak, James P. (1); Tyurina, Yulia Y.; Tyurin, Vladimir A.; Lazo, John S.; Kagan, Valerian E.
CS (1) Dep. Environ. Occupational Health Sch. Public Health, RIDC Park, 260 Kappa Drive, Univ. Pittsburgh, Pittsburgh, PA 15236 USA
SO Biochemistry, (Sept. 29, 1998) Vol. 37, No. 39, pp. 13781-13790.
ISSN: 0006-2960.
DT Article
LA English
AB The formation of reactive oxygen species has been associated with **apoptosis**. To assess the role of lipid peroxidation in **apoptosis**, we used 2,2'-azobis(2,-4-dimethylisovaleronitrile) (AMVN) to generate peroxyl radicals within cellular membranes of HL-60 cells. cis-Parinaric acid (cis-PnA) metabolically integrated into phospholipids of HL-60 cells was used as a probe to assess the extent of lipid peroxidation within specific phospholipid classes. Within 2 h, AMVN (500 muM) randomly oxidized more than 85% of cis-PnA contained in all

major classes of phospholipids. AMVN-induced lipid peroxidation was followed by **apoptosis** as determined by nuclear condensation, DNA fragmentation, and **annexin** V binding to externalized **phosphatidylserine** (PS). Fluorescamine derivatization of external aminophospholipids revealed that PS, but not phosphatidylethanolamine, was externalized. The vitamin E analogue, 6-hydroxy-2,2,5,7,8-pentamethylchromane (PMC), inhibited overall oxidation of cis-PnA in phospholipids by more than 85%. Not all phospholipids, however, were equally protected. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and sphingomyelin were nearly completely protected by PMC, while oxidation of PS was unaffected in whole living cells. The insensitivity of PS to PMC was not an intrinsic property because PMC protected all lipids equally during AMVN oxidation of liposomes prepared from cis-PnA-labeled cells. The potential role for PS oxidation in **apoptosis** was further suggested by the faithful execution of **apoptosis** following coexposure of cells to AMVN and PMC.

L9 ANSWER 139 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:365404 BIOSIS
DN PREV199800365404
TI Induction of **apoptosis** in plasmacytoma cells by a cytotoxic factor secreted by P388D1 macrophage-like cell line.
AU Chu, C.-Y.; Liu, T.-H.; Tseng, J. (1)
CS (1) Dep. Biol., Natl. Taiwan Normal Univ., 88 Ting-Chou Rd., Sect. 4, Taipei 117 Taiwan
SO International Journal of Immunotherapy, (1998) Vol. 14, No. 2, pp. 69-81. ISSN: 0255-9625.
DT Article
LA English
AB Tumoricidal activity is one of the major effector functions of activated macrophages. A previous study of ours demonstrated that the culture supernatant of P388D, murine macrophage-like cells show a cytotoxic effect on plasmacytoma MOPC-315, MPC-11 and myeloma FO but have no effect on J558 myeloma cells. In this study, the plasmacytoma cytotoxic factor in P388D1 culture supernatant was partially purified by a DEAE-Sephacel ionic-exchanger chromatography and a panel of monoclonal antibodies against plasmacytoma cytotoxic factor was prepared. All monoclonal antibodies partially blocked the P388D1-mediated tumoricidal activity. A large-scale purification was performed by ammonium sulfate fractionation (40-60% saturation), followed by an immunoaffinity chromatography using one of the antiplasmacytoma cytotoxic factor monoclonal antibodies, CB7-C2. The affinity-purified plasmacytoma cytotoxic factor had IC50 at 3.11 mug/ml for 2 X 104 MOPC-315 cells and showed a major band with an estimated molecular weight of 62 kDa on SDS-PAGE gel. However, CB7.C2 recognized a single band with an estimated molecular weight of 120-130 kDa on Western blotting, suggesting that the native form of plasmacytoma cytotoxic factor could be a dimer. Plasmacytoma cytotoxic factor-mediated cytotoxicity involved **apoptosis**. Data from both agarose gel electrophoresis and terminal deoxynucleotidyl transferase-mediated deoxyuridine 5-triphosphate (dUTP) nick-end-labeling method indicated that a significant amount of DNA fragmentation was induced in plasmacytoma cytotoxic factor-treated MOPC-315 cells. Using an **Annexin** V staining technique, the plasmacytoma cytotoxic factor-induced **apoptosis** was confirmed further by observing the **phosphatidylserine** redistribution on the plasma membrane of plasmacytoma cytotoxic factor-treated cells. The plasmacytoma cytotoxic factor-induced **apoptosis** was dose-dependent and time-dependent and could be neutralized by CB7.C2 antiplasmacytoma cytotoxic factor antibody. Taken together, our studies demonstrate that a 62 kDa protein secreted by P388D1 macrophage-like cells shows its cytotoxic effect on MOPC-315 plasmacytoma cells via induction of **apoptosis**.

L9 ANSWER 140 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:360561 BIOSIS

DN PREV199800360561
TI Surface exposure of **phosphatidylserine** during **apoptosis** of rat thymocytes precedes nuclear changes.
AU Stuart, Marc C. A.; Damoiseaux, Jan G. M. C.; Frederik, Peter M. (1); Arends, Jan-Willem; Reutelingsperger, Chris P. M.
CS (1) Electron Microscopy Unit, Dep. Pathol., Maastricht Univ., P.O. Box 616, 6200 MD Maastricht Netherlands
SO European Journal of Cell Biology, (May, 1998) Vol. 76, No. 1, pp. 77-83. ISSN: 0171-9335.
DT Article
LA English
AB Cell surface exposure of **phosphatidylserine** (PS) during **apoptosis** serves recognition and removal of the dying cell by phagocytes. Loss of phospholipid asymmetry and PS exposure is investigated by immunocytochemistry and related to morphological changes. Loss of membrane asymmetry was determined on dexamethasone-treated rat thymocytes using the PS specific probe **annexin** V. Thymocytes incubated in the presence of dexamethasone were studied in time series during the execution of the apoptotic program. Thymocytes first start to expose PS at their cell surface. At this initial stage the barrier function of the plasma membrane remains intact. At a later stage the plasma membrane becomes leaky for compounds like propidium iodide and subsequently the cell disintegrates into apoptotic bodies. Microscopical evaluation of dexamethasone-treated thymocytes showed that the cells with an apoptotic morphology all bound **annexin** V. The cells with a normal viable morphology lacked **annexin** V binding except for those cells that started to shed small vesicles. These vesicles were positive for **annexin** V, indicating a local disturbance of the phospholipid asymmetry. The local exposure of PS is considered to be a very early event of **apoptosis**, preceding the full sequence of morphological changes at the ultrastructural level.

L9 ANSWER 143 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:341473 BIOSIS
DN PREV199800341473
TI Preparation and characterization of an endogenously fluorescent **annexin** for detection of apoptotic cells.
AU Ernst, Joel D. (1); Yang, Lin; Rosales, Jesusa L.; Broaddus, V. Courtney
CS (1) Div. Infect. Dis., Rosalind Russell Arthritis Res. Lab., Univ. Calif., San Francisco, CA 94143 USA
SO Analytical Biochemistry, (June 15, 1998) Vol. 260, No. 1, pp. 18-23. ISSN: 0003-2697.
DT Article
LA English
AB **Annexin** proteins specifically bind anionic phospholipids such as **phosphatidylserine**, which are normally confined to the cytoplasmic leaflet of cellular membranes. During programmed cell death, or **apoptosis**, this phospholipid asymmetry is lost, and anionic phospholipids are exposed on the extracellular leaflet of the plasma membrane where they are accessible to exogenously added, labeled **annexins**. Chemically (e.g., fluorescein isothiocyanate (FITC))-modified **annexin** V has been widely used to detect and enumerate apoptotic cells by flow cytometry. We prepared chimeric proteins containing green fluorescent protein (GFP) fused to **annexin** V. A chimera containing GFP fused to the C-terminus of **annexin** V was soluble and fluorescent, but was unable to bind phospholipids. In contrast, a chimera containing GFP fused to the N-terminus of **annexin** V specifically bound apoptotic cells. GFP-**annexin** V represents a sensitive and facile alternative to FITC-**annexin** V for studies of **apoptosis**.

L9 ANSWER 144 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:337533 BIOSIS
DN PREV199800337533

TI Effect of cyclosporine therapy on the intensity of **apoptosis** in a model of heterotopic heart transplant: Evaluation with Tc-99m **annexin** V lipocortin (TcAVL) imaging.
AU Blankenberg, F. (1); Ohtsuki, K. (1); Tait, J.; Berry, G. (1); Davis, E. (1); Vriens, P. (1); Stoot, J. (1); Hoyt, G. (1); Robbins, R. (1); Kopiwoda, S. (1); Strauss, H. W. (1)
CS (1) Stanford Univ. Hosp., Stanford, CA USA
SO Journal of Nuclear Medicine, (May, 1998) Vol. 39, No. 5 SUPPL., pp. 160P. Meeting Info.: 45th Annual Meeting of the Society of Nuclear Medicine Toronto, Ontario, Canada June 7-11, 1998 Society of Nuclear Medicine . ISSN: 0161-5505.
DT Conference
LA English

L9 ANSWER 145 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:321801 BIOSIS
DN PREV199800321801
TI Flow cytometric assessment of three different methods for the measurement of in vitro **apoptosis**.
AU Pepper, Chris; Thomas, Alun; Tucker, Heather; Hoy, Terry; Bentley, Paul (1)
CS (1) Dep. Haematol., Llandough Hosp., Penarth, South Glamorgan UK
SO Leukemia Research, (May, 1998) Vol. 22, No. 5, pp. 439-444. ISSN: 0145-2126.
DT Article
LA English
AB Chlorambucil-induced **apoptosis** was assessed by three different flow cytometric methods in B-cell chronic lymphocytic leukaemia (B-CLL) cells cultured in vitro and the results were compared with those derived from the morphological assessment of the same samples. Spontaneous **apoptosis** was consistently observed in the control cultures in the absence of drug but this accounted for less than 12% of all cells in every case. The methods under investigation were the **Annexin** V labelling assay, the terminal deoxynucleotidyl transferase (TdT) end-labelling assay and the labelling of a 38 kDa mitochondrial membrane protein (7A6 antigen) which is exposed on cells undergoing apoptotic cell death (Apo2.7 assay). The **Annexin** V assay consistently stained a higher percentage of cells and with a greater separation between the positive and negative cell populations. We conclude that the **phosphatidyl serine** translocation to the outer leaflet of the cell membrane following an apoptotic signal, as labelled by **Annexin** V, probably occurs before the development of the DNA strand breaks or the exposure of 7A6 antigen in those cells triggered to die by **apoptosis**.

L9 ANSWER 147 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:310966 BIOSIS
DN PREV199800310966
TI In vivo detection and imaging of **phosphatidylserine** expression during programmed cell death.
AU Blankenberg, Francis G. (1); Katsikis, Peter D.; Tait, Jonathan F.; Davis, R. Eric; Naumovski, Louis; Ohtsuki, Katsuichi; Kopiwoda, Susan; Abrams, Michael J.; Darkes, Marilyn; Robbins, Robert C.; Maecker, Holden T.; Strauss, H. W.
CS (1) Dep. Radiol., Stanford Univ. Sch. Med., 300 Pasteur Dr., Stanford, CA 94305-5105 USA
SO Proceedings of the National Academy of Sciences of the United States of America, (May 26, 1998) Vol. 95, No. 11, pp. 6349-6354. ISSN: 0027-8424.
DT Article
LA English
AB One of the earliest events in programmed cell death is the externalization of **phosphatidylserine**, a membrane phospholipid normally restricted to the inner leaflet of the lipid bilayer. **Annexin** V,

an endogenous human protein with a high affinity for membrane bound **phosphatidylserine**, can be used in vitro to detect **apoptosis** before other well described morphologic or nuclear changes associated with programmed cell death. We tested the ability of exogenously administered radiolabeled **annexin** V to concentrate at sites of apoptotic cell death in vivo. After derivatization with hydrazinonicotinamide, **annexin** V was radiolabeled with technetium 99m. In vivo localization of technetium 99m hydrazinonicotinamide-**annexin** V was tested in three models: fuminant hepatic **apoptosis** induced by anti-Fas antibody injection in BALB/c mice; acute rejection in ACI rats with transplanted heterotopic PVG cardiac allografts; and cyclophosphamide treatment of transplanted 38C13 murine B cell lymphomas. External radionuclide imaging showed a two- to sixfold increase in the uptake of radiolabeled **annexin** V at sites of **apoptosis** in all three models. Immunohistochemical staining of cardiac allografts for exogenously administered **annexin** V revealed intense staining of numerous myocytes at the periphery of mononuclear infiltrates of which only a few demonstrated positive apoptotic nuclei by the terminal deoxynucleotidyltransferase-mediated UTP end labeling method. These results suggest that radiolabeled **annexin** V can be used in vivo as a noninvasive means to detect and serially image tissues and organs undergoing programmed cell death.

L9 ANSWER 153 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:185032 BIOSIS
DN PREV199800185032
TI Ex vivo evidence of lymphocyte **apoptosis** in hairy cell leukemia, induced by 2-chlorodeoxyadenosine treatment.
AU Idink-Mecking, C. A. M.; Richel, D. J.; Vermes, I. (1); Schaafsma, M. R.; Reutelingsperger, C.; Haanen, C.
CS (1) Dep. Clinical Chem., Med. Spectrum Twente, P.O. Box 50.000, 7500 KA Enschede Netherlands
SO Annals of Hematology, (Jan., 1998) Vol. 76, No. 1, pp. 25-29. ISSN: 0939-5555.
DT Article
LA English
AB In all living cells **phosphatidylserine** (PS) is located at the cytosol side of the membrane and becomes exposed at the cell surface only during necrosis or **apoptosis**. This phenomenon allows measurement of cell death on a cell-by-cell basis, using labeled **Annexin** V, which has a strong affinity to PS. Two patients with hairy cell leukemia (HCL) who had relapsed after splenectomy and alpha-interferon therapy were treated with 2-chlorodeoxyadenosine (2-CdA) for 7 days. Blood samples were taken from the start of therapy until day 22. Percentages of HCL cells, T cells, B cells, and NK cells were measured with PE-labeled monoclonal antibodies by flow cytometry (FCM). The absolute lymphocyte count dropped rapidly to almost zero in both patients within 7 days. The disappearance rate of lymphocyte subfractions did not show a specific pattern. The percentage of **apoptosis** in lymphocyte subfractions was measured in freshly prepared cell samples by FCM with FITC-labeled **Annexin** V in the propidium iodide-negative (non-necrotic) cell fraction. Percentages of PS-positive cells increased gradually till a nadir of **Annexin** V positivity was reached at 14 and 16 days. Because during the first week the absolute cell counts became almost zero, the absolute numbers of PS-positive cells were still extremely low, i.e., less than 0.1 X 109/l. Nevertheless, we observed apoptotic cells in circulation after 2-CdA therapy. To our knowledge, this is the first report of the occurrence of **apoptosis** ex vivo in circulating blood cells after cytotoxic therapy.

L9 ANSWER 159 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:94039 BIOSIS
DN PREV199800094039

TI Strategies for phenotyping apoptotic peripheral human lymphocytes comparing ISNT, **annexin**-V and 7-AAD cytofluorometric staining methods.
AU Lecoeur, Herve; Ledru, Eric; Prevost, Marie-Christine; Gougeon, Marie-Lise (1)
CS (1) Unite d'Oncol. Virale, Dep. SIDA Retrovirus, Inst. Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15 France
SO Journal of Immunological Methods, (Dec. 1, 1997) Vol. 209, No. 2, pp. 111-123.
ISSN: 0022-1759.
DT Article
LA English
AB The present article compares the reliability of four previously described cytofluorometric methods of **apoptosis** quantification for phenotyping apoptotic human lymphocytes. Each of these assays detects distinct cellular alterations of the apoptotic process. Alteration in plasma membrane integrity can be evaluated following 7-AAD incorporation and the translocation of **phosphatidylserine** from the inner to the outer layer of the plasma membrane can be detected through the FITC-**annexin** V staining. DNA strand breaks in apoptotic nuclei can be evidenced by the ISNT assay and finally morphological modifications can be followed with FSC/SSC criteria. Comparative analysis of **apoptosis** in cultured PBMC from HIV-infected patients considering the FSC/SSC parameters, 7-AAD stainability and **annexin** V fixation revealed that the latter identifies early apoptotic cells, also characterized as 7-AADlow with a reduced FSC. Moreover these three methods proved to be reliable and gave statistically similar results when combined with cell surface detection of antigens such as CD4, CD8 and CD19 by specific mAbs. Importantly, the 7-AAD assay easily allowed the identification of debris/apoptotic bodies, which were still stained by anti-cell surface mAbs and might therefore significantly distort the **apoptosis** percentage in a given lymphocyte subset. In the present report we also point out that the ISNT assay is not appropriate for phenotyping apoptotic lymphocytes in PBMC. Indeed it can particularly underestimate the rate of **apoptosis** in the B-cell subset. This was found to be related to the **apoptosis**-associated decrease in cell surface antigen expression, which is dramatically exacerbated in the ISNT assay because of the stripper effect of ethanol used for cell permeabilization. Finally, we propose a three step analytical strategy to accurately phenotype apoptotic peripheral human lymphocytes. It includes two gating steps performed on FSC/SSC criteria and 7-AAD/FSC parameters to eliminate monocytes, granulocytes and debris-apoptotic bodies, the third step being the phenotyping step itself, performed in dual or triple staining experiments. Altogether these observations emphasize that it is essential to assess critically the ability of a cytofluorometric method to phenotype apoptotic cells in complex lymphoid populations and that inaccurate identification of cell subsets undergoing **apoptosis** can be readily overcome by gating properly the lymphoid population, and using assays which preserve cell surface structure.

L9 ANSWER 160 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:90453 BIOSIS
DN PREV199800090453
TI **Annexin** V-affinity assay: A review on an **apoptosis** detection system based on **phosphatidylserine** exposure.
AU van Engeland, Manon; Nieland, Luc J. W.; Ramaekers, Frans C. S.; Schutte, Bert (1); Reutelingsperger, Chris P. M.
CS (1) Dep. Mol. Cell Biol. Genetics, Maastricht Univ., PO Box 616, 6200 MD Maastricht Netherlands
SO Cytometry, (Jan., 1998) Vol. 31, No. 1, pp. 1-9.
ISSN: 0196-4763.
DT General Review
LA English
AB **Apoptosis** is a programmed, physiological mode of cell death that

plays an important role in tissue homeostasis. Understanding of the basic mechanisms that underlie **apoptosis** will point to potentially new targets of therapeutic treatment of diseases that show an imbalance between cell proliferation and cell loss. In order to conduct such research, techniques and tools to reliably identify and enumerate death by **apoptosis** are essential. This review focuses on a novel technique to detect **apoptosis** by targeting for the loss of phospholipid asymmetry of the plasma membrane. It was recently shown that loss of plasma membrane asymmetry is an early event in **apoptosis**, independent of the cell type, resulting in the exposure of **phosphatidylserine** (PS) residues at the outer plasma membrane leaflet. **Annexin** V was shown to interact strongly and specifically with PS and can be used to detect **apoptosis** by targeting for the loss of plasma membrane asymmetry. Labeled **annexin** V can be applied both in flow cytometry and in light microscopy in both vital and fixed material by using appropriate protocols. The **annexin** V method is an extension to the current available methods. This review describes the basic mechanisms underlying the loss of membrane asymmetry during **apoptosis** and discusses the novel **annexin** V-binding assay.

L9 ANSWER 169 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1997:481020 BIOSIS
DN PREV199799780223
TI Early **phosphatidylserine** membrane translocation in staurosporine-induced **apoptosis** of rat ventricular myocytes.
AU Hatem, Stephane (1); Ruecker-Martin, Catherine; Henaff, Morgana; Planche, Claude; Mercadier, Jean-Jacques
CS (1) INSERM U460, Faculte X. Bichat, Paris France
SO European Heart Journal, (1997) Vol. 18, No. ABSTR. SUPPL., pp. 611. Meeting Info.: XIXth Congress of the European Society of Cardiology together with the 32nd Annual General Meeting of the Association of European Paediatric Cardiologists (AEPC) Stockholm, Sweden August 24-28, 1997
ISSN: 0195-668X.
DT Conference; Abstract; Conference
LA English

L9 ANSWER 170 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1997:460790 BIOSIS
DN PREV199799759993
TI **Annexin**-V and TUNEL use in monitoring the progression of **apoptosis** in plants.
AU O'Brien, Iona E. W. (1); Reutelingsperger, Chris P. M.; Holdaway, Karen M.
CS (1) Mount Albert Res. Centre, HortResearch, Private Bag 92169, Auckland New Zealand
SO Cytometry, (1997) Vol. 29, No. 1, pp. 28-33.
ISSN: 0196-4763.
DT Article
LA English
AB An early indicator of **apoptosis** in mammalian cells is the loss of the phospholipid membrane asymmetry of the cell. This results in exposure of **phosphatidylserine** on the outer surface of the plasma membrane. This change in membrane asymmetry can be analysed using **annexin** V. A further feature of **apoptosis**, DNA breaks, can be measured by the TUNEL assay. Using flow cytometry, we have identified both of these features in HL-60 cells and by modifying the techniques for plants, we have verified that these features also occur in plant cells undergoing **apoptosis**. In both plant and HL-60 cells, **apoptosis** was induced by treatment with camptothecin (1 mu-M). **Annexin** V binding was found to be an early indicator of **apoptosis**, occurring prior to the detection of DNA strand breaks as monitored by the TUNEL assay. In plant cells, chromatin condensation was detected prior to the detection of **annexin** V. No loss in

membrane integrity occurred with apoptotic cells in comparison with necrotic cells. Our findings indicate that a form of **apoptosis** occurs in plants, with flow cytometric characteristics similar to those of **apoptosis** in HL-60 cells.

L9 ANSWER 171 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1997:450480 BIOSIS
DN PREV199799749683
TI Early detection of **apoptosis** using a fluorescent conjugate of **annexin** V.
AU Zhang, Guohong; Gurtu, Vanessa; Kain, Steven R.; Yan, Guochen
CS CLONTECH Lab. Inc., 1020 East Meadow Circle, Palo Alto, CA 94303 USA
SO Biotechniques, (1997) Vol. 23, No. 3, pp. 525-526, 528-531. ISSN: 0736-6205.
DT Article
LA English
AB **Apoptosis** of mammalian cells is accompanied by various morphological changes including nuclear condensation, DNA fragmentation and cell surface changes. Methods developed over the past few years have focused on detection of DNA-associated changes that occur rather late in **apoptosis**. However detection of **apoptosis** at early stages, before gross morphological changes, is critical for understanding the pathways of programmed cell death. In this report, we describe a rapid and reliable assay for detecting early stages of **apoptosis**. This assay is based on the observation that soon after initiating **apoptosis**, most mammalian cell types translocate **phosphatidylserine** (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS can be specifically detected by staining with fluorescein isothiocyanate (FITC)-labeled **annexin** V (**annexin** V-FITC), a protein with a strong, natural affinity for PS. Using this assay, we have detected apoptotic cells in culture, in real time, using fluorescence microscopy and flow cytometry. In combination with vital dye staining, the progressive stages of **apoptosis** were observed PS redistribution occurs earlier than DNA-associated changes and membrane leakage. In addition, PS externalization occurs during **apoptosis** induced by a variety of stimuli. Therefore, the **annexin** V binding assay provides an excellent indicator for the early stages of **apoptosis**.

L9 ANSWER 174 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1997:363657 BIOSIS
DN PREV199799655590
TI **Apoptosis** and secondary necrosis of lymphocytes in culture.
AU Vermes, I. (1); Haanen, C.; Richel, D. J.; Schaafsma, M. R.; Kalsbeek-Batenburg, E.; Reutelingsperger, C. P. M.
CS (1) Dep. Clinical Chem., Med. Spectum Twente, Hosp. Group, PO Box 5000, NL-7500 KA Enschede Netherlands
SO Acta Haematologica (Basel), (1997) Vol. 98, No. 1, pp. 8-13. ISSN: 0001-5792.
DT Article
LA English
AB It has been reported that cultured peripheral B lymphocytes of chronic lymphocytic leukemia (B-CLL) patients show a high degree of **apoptosis** (programmed cell death). Till now, no data exist about the occurrence of in vitro **apoptosis** of normal B and T cells. We measured the amount of **apoptosis** and secondary necrosis (type 2 necrosis) in B-CLL lymphocytes and in normal peripheral B and T lymphocytes in culture. Observations were made on BCLL lymphocytes and on normal B and T cells purified by immunomagnetic cell sorting. **Apoptosis** and secondary necrosis were measured using a recently described sensitive flow-cytometric assay, probing simultaneously for cell surface exposure of **phosphatidylserine** with the use of FITC-labeled **annexin**-V and for cell membrane integrity as demonstrated by the exclusion of propidium iodide. The degree of in vitro

apoptosis and secondary necrosis of normal B cells appears to be higher than that of normal T cells, and even higher than that of B-CLL cells. The results indicate that cultured mature circulating normal B lymphocytes exhibit a higher cell death rate than normal T cells and B-CLL lymphocytes.

L9 ANSWER 182 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1997:97307 BIOSIS
DN PREV199799396510
TI Quantification of **apoptosis** in solid human tumors by **phosphatidylserine** expression at the external surface of the plasma membrane.
AU Hofs, H. P.; Bakker, P. J.; Aten, J. A.
CS Academic Medical Cent., Univ. Amsterdam, Amsterdam Netherlands
SO Molecular Biology of the Cell, (1996) Vol. 7, No. SUPPL., pp. 517A. Meeting Info.: Annual Meeting of the 6th International Congress on Cell Biology and the 36th American Society for Cell Biology San Francisco, California, USA December 7-11, 1996
ISSN: 1059-1524.
DT Conference; Abstract; Conference
LA English

L9 ANSWER 191 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1995:544074 BIOSIS
DN PREV199698558374
TI Early redistribution of plasma membrane **phosphatidylserine** is a general feature of **apoptosis** regardless of the initiating stimulus: Inhibition by overexpression of Bcl-2 and Ab1.
AU Martin, Seamus J. (1); Reutelingsperger, Chris P. M.; McGahon, Anne J.; Rader, James A.; Van Schie, Rob C. A. A.; Laface, Drake M.; Green, Douglas R.
CS (1) Div. Cellular Immunol., La Jolla Inst. Allergy Immunology, 11149 N. Torrey Pines Road, La Jolla, CA 92037 USA
SO Journal of Experimental Medicine, (1995) Vol. 182, No. 5, pp. 1545-1556.
ISSN: 0022-1007.
DT Article
LA English
AB A critical event during programmed cell death (PCD) appears to be the acquisition of plasma membrane (PM) changes that allows phagocytes to recognize and engulf these cells before they rupture. The majority of PCD seen in higher organisms exhibits strikingly similar morphological features, and this form of PCD has been termed **apoptosis**. The nature of the PM changes that occur on apoptotic cells remains poorly defined. In this study, we have used a **phosphatidylserine** (PS)-binding protein (**annexin** V) as a specific probe to detect redistribution of this phospholipid, which is normally confined to the inner PM leaflet, during **apoptosis**. Here we show that PS externalization is an early and widespread event during **apoptosis** of a variety of murine and human cell types, regardless of the initiating stimulus, and precedes several other events normally associated with this mode of cell death. We also report that, under conditions in which the morphological features of **apoptosis** were prevented (macromolecular synthesis inhibition, overexpression of Bcl-2 or Abl), the appearance of PS on the external leaflet of the PM was similarly prevented. These data are compatible with the notion that activation of an inside-outside PS translocase is an early and widespread event during **apoptosis**.

L9 ANSWER 192 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1995:405596 BIOSIS
DN PREV199598419896
TI A novel assay for **apoptosis** flow cytometric detection of **phosphatidylserine** expression on early apoptotic cells using fluorescein labelled **Annexin** V.

AU Vermes, Istvan (1); Haanen, Clemens; Steffens-Nakken, Helga; Reutelingsperger, Chris
CS (1) Dep. Clin. Chem., Med. Spectrum Twente, P.O. Box 50.000, 7500 KA Enschede Netherlands
SO Journal of Immunological Methods, (1995) Vol. 184, No. 1, pp. 39-51. ISSN: 0022-1759.
DT Article
LA English
AB In the early stages of **apoptosis** changes occur at the cell surface, which until now have remained difficult to recognize. One of these plasma membrane alterations is the translocation of **phosphatidylserine** (PS) from the inner side of the plasma membrane to the outer layer, by which PS becomes exposed at the external surface of the cell. **Annexin** V is a Ca-2+ dependent phospholipid-binding protein with high affinity for PS. Hence this protein can be used as a sensitive probe for PS exposure upon the cell membrane. Translocation of PS to the external cell surface is not unique to **apoptosis**, but occurs also during cell necrosis. The difference between these two forms of cell death is that during the initial stages of **apoptosis** the cell membrane remains intact, while at the very moment that necrosis occurs the cell membrane looses its integrity and becomes leaky. Therefore the measurement of **Annexin** V binding to the cell surface as indicative for **apoptosis** has to be performed in conjunction with a dye exclusion test to establish integrity of the cell membrane. This paper describes the results of such an assay, as obtained in cultured HSB-2 cells, rendered apoptotic by irradiation and in human lymphocytes, following dexamethasone treatment. Untreated and treated cells were evaluated for **apoptosis** by light microscopy, by measuring the amount of hypo-diploid cells using of DNA flow cytometry (FCM) and by DNA electrophoresis to establish whether or not DNA fragmentation had occurred. **Annexin** V binding was assessed using bivariate FCM, and cell staining was evaluated with fluorescein isothiocyanate (FITC)-labelled **Annexin** V (green fluorescence), simultaneously with dye exclusion of propidium iodide (PI) (negative for red fluorescence). The test described, discriminates intact cells (FITC-/PI-), apoptotic cells (FITC+/PI-) and necrotic cells (FITC+/PI+). In comparison with existing traditional tests the **Annexin** V assay is sensitive and easy to perform. The **Annexin** V assay offers the possibility of detecting early phases of **apoptosis** before the loss of cell membrane integrity and permits measurements of the kinetics of apoptotic death in relation to the cell cycle. More extensive FCM will allow discrimination between different cell subpopulations, that may or may not be involved in the apoptotic process.

L9 ANSWER 194 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1995:333428 BIOSIS
DN PREV199598347728
TI A novel assay for **apoptosis** based upon flow cytometric detection of **phosphatidylserine** on the cell surface with use of FITC-labelled **Annexin** V.
AU Vermes, Istvan (1); Haanen, Clemens (1); Reutelingsperger, Chris
CS (1) Dep. Clinical Chem., Med. Spectrum Twente, Enschede Netherlands
SO Clinical Chemistry, (1995) Vol. 41, No. S6 PART 2, pp. S91. Meeting Info.: 47th Annual Meeting of the American Association for Clinical Chemistry, Inc. Anaheim, California, USA July 16-20, 1995 ISSN: 0009-9147.
DT Conference
LA English

L9 ANSWER 195 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1995:119572 BIOSIS
DN PREV199598133872
TI Human neutrophils lose their surface Fc-gamma-RIII and acquire **annexin** V binding sites during **apoptosis** in vitro.

AU Homburg, Christa H. E.; De Haas, Masja; Von Dem Borne, Albert E. G. K; Verhoeven, Arthur J.; Reutelingsperger, Chris P. M.; Roos, Dirk (1)
CS (1) Central Lab., Neth. Red Cross Blood Transfusion Serv., Plesmanlaan 125, 1066 CX Amsterdam Netherlands
SO Blood, (1995) Vol. 85, No. 2, pp. 532-540.
ISSN: 0006-4971.
DT Article
LA English
AB We have previously reported that neutrophilic granulocytes rapidly release part of their Fc-gamma-RIII from the plasma membrane upon in vitro activation, probably by proteolytic cleavage. In plasma and other body fluids, released or soluble Fc-gamma-RIII has been found in considerable amounts. In the present study, neutrophils were kept in maintenance culture for 18 to 24 hours. Forty percent of the neutrophils completely lost Fc-gamma-RIII, and the remainder of the cells showed a 60% decrease in Fc-gamma-RIII expression on their surface. Released Fc-gamma-RIII was detected in the culture supernatant. Nevertheless, more than 90% of the cells was viable as judged by hydrolysis of fluorescein diacetate. The presence of interferon gamma, granulocyte colony-stimulating factor, or granulocyte-macrophage colony-stimulating factor, but not interleukin-3 (IL-3), IL-6, or IL-8, in the culture medium increased the number of cells that still expressed Fc-gamma-RIII. We found that this loss of Fc-gamma-RIII was not the result of cell activation but correlated strongly with **apoptosis**. The Fc-gamma-RIII-negative subpopulation exhibited typical morphologic changes, such as nuclear condensation and DNA fragmentation. Furthermore, this subpopulation appeared to have acquired the property of binding **Annexin** V, a calcium-dependent, phospholipid-binding protein with high affinity for **phosphatidylserine**. The external exposure of this phospholipid by cells has been reported to occur during **apoptosis**. The property of **Annexin** V binding was not shared by the nonapoptotic, Fc-gamma-RIII-positive subpopulation. In this respect, we identified binding of **Annexin** V as an convenient marker for apoptotic cells. Our results indicate that soluble Fc-gamma-RIII in body fluids might be derived for a large part from neutrophils undergoing **apoptosis** in the tissues.

L9 ANSWER 196 OF 197 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1995:55501 BIOSIS
DN PREV199598069801
TI **Annexin** V for flow cytometric detection of **phosphatidylserine** expression on B cells undergoing **apoptosis**.
AU Van Oers, M. H. J. (1); Reutelingsperger, C. P. M.; Kuyten, G. A. M.; Von Dem Borne, A. E. G. K.; Koopman, G.
CS (1) Dep. Haematol., Acad. Med. Cent., Univ. Amsterdam, Amsterdam Netherlands
SO Blood, (1994) Vol. 84, No. 10 SUPPL. 1, pp. 291A.
Meeting Info.: Abstracts Submitted to the 36th Annual Meeting of the American Society of Hematology Nashville, Tennessee, USA December 2-6, 1994
ISSN: 0006-4971.
DT Conference
LA English

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=> d his

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     FILE 'USPATFULL' ENTERED AT 09:46:02 ON 10 SEP 2003
L1         10735 S APOPTOSIS
L2          2901 S PHOSPHATIDYLSERINE
L3          1209 S PHOSPHATIDYL SERINE
L4          3860 S L2 OR L3
L5           620 S L1 AND L4
L6          1157 S ANNEXIN
L7           306 S L6 AND L5

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COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    63.03      63.24

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L2          9155 S PHOSPHATIDYLSERINE
L3          2238 S PHOSPHATIDYL SERINE
L4         10447 S L2 OR L3
L5          1028 S L4 AND L1
L6          4990 S ANNEXIN
L7           361 S L5 AND L6
L8       1343801 S PD>2000
L9           197 S L7 NOT L8

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L10          917 S L5
L11       931105 S ANST/RL
L12           67 S L10 AND L11

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FULL ESTIMATED COST                                   121.13     294.86

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                      ENTRY    SESSION
CA SUBSCRIBER PRICE                                   -22.94     -22.94

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STN INTERNATIONAL SESSION SUSPENDED AT 15:27:31 ON 09 SEP 2003
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